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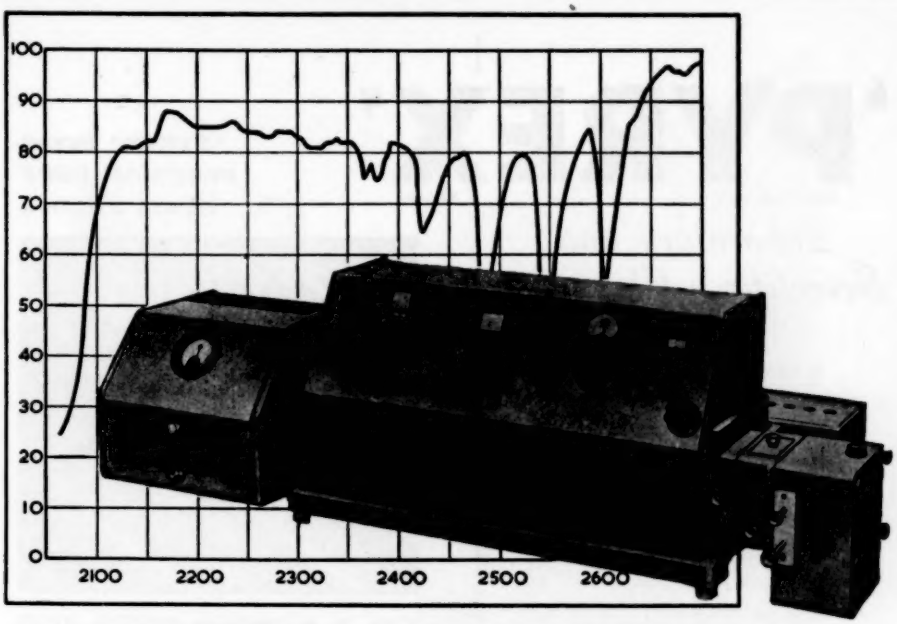


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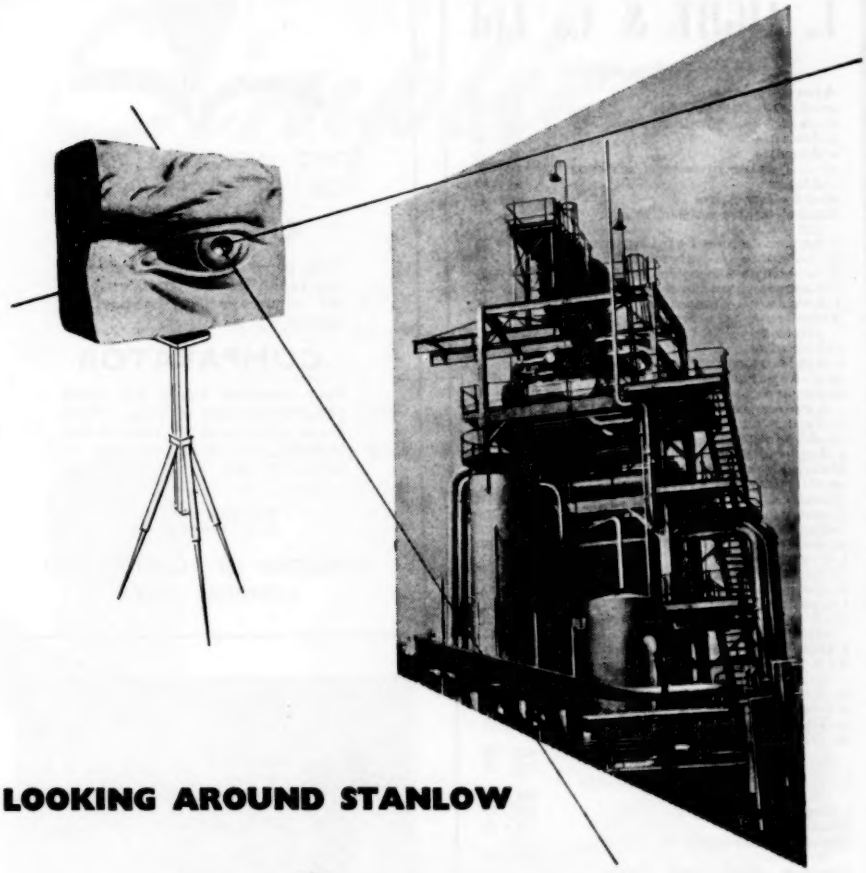

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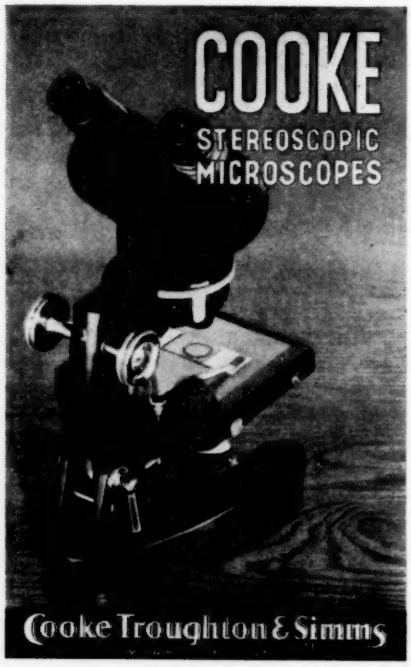

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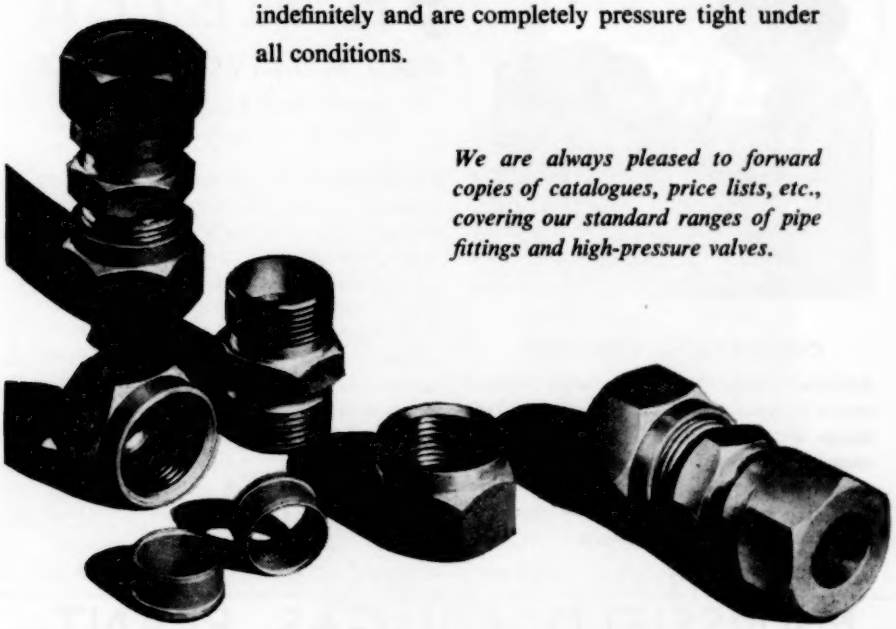

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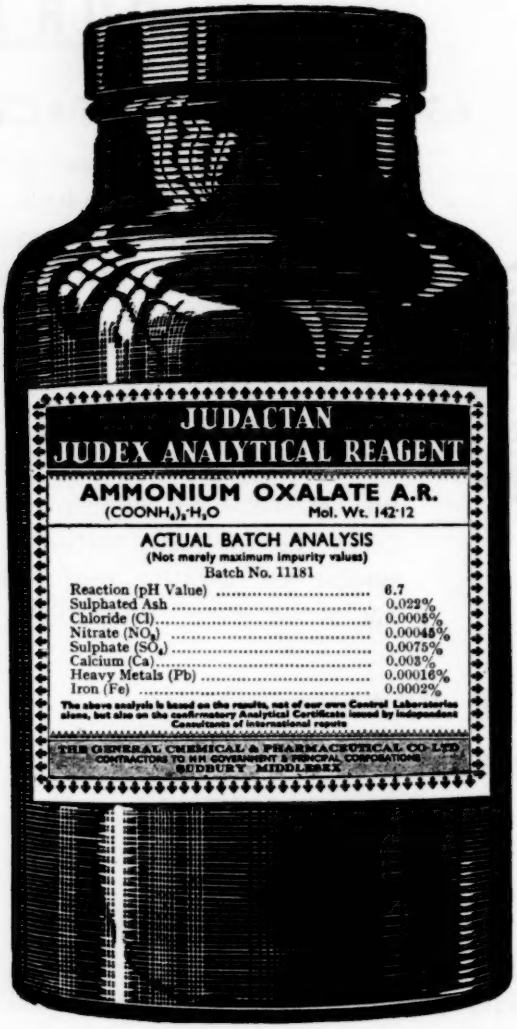
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## 564. *Determinations of Electrokinetic Charge and Potential by the Sedimentation Method. Part II.* Pyrex Glass in Aqueous Potassium Chloride Solutions.*

By G. A. H. Elton and F. G. Hirschler.

The sedimentation velocity method for the determination of electrokinetic charges and potentials is applied to the study of Pyrex-glass surfaces in dilute aqueous potassium chloride solutions, (*a*) Pyrex powder, consisting of irregularly shaped particles, and (*b*) microspheres being used. The good agreement obtained between results with these different particles is further support for the validity of the sedimentation equation for non-spherical particles. The presence of a small amount of silver chloride in the solutions is shown to produce, for low potassium chloride concentrations, a considerable increase in charge, confirming previous conclusions on the unsuitability of silver–silver chloride electrodes for electrokinetic use.

In connection with an experimental study of the surface conductivity of Pyrex-glass surfaces in aqueous potassium chloride solutions, to be described elsewhere, it became necessary to determine the electrokinetic charge $\sigma$ carried by Pyrex surfaces in such solutions. This has been done by using the method of electroviscous sedimentation (see Elton, *J. Chem. Physics*, 1951, **19**, 1317; Dulin and Elton, Part I *) and the opportunity has been taken of further testing the theory of the method by comparing results obtained with irregularly shaped particles of ground Pyrex with those using Pyrex microspheres prepared by the method of Bloomquist and Clark (*Ind. Eng. Chem. Anal.*, 1940, **12**, 61). In addition, since some of the surface conductance measurements had involved the use of silver–silver chloride reversible electrodes, it was necessary to assess the effect of any dissolution of silver chloride from these electrodes on the charge carried by the Pyrex surface. For this reason, two sets of sedimentation experiments were carried out, (i) plain potassium chloride solutions, and (ii) potassium chloride solutions saturated with respect to silver chloride being used.

Calculation of the electrokinetic charges from the sedimentation velocity results for non-spherical particles was made, as in Part I, from the equation

$$\sigma^2 = g\kappa(\rho_2 - \rho_1)(u_0 - u)/MA_0{}^2\rho_2 u u_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where $g$ is the gravitational constant, $\rho_2$ and $\rho_1$ are the densities of glass and solution respectively, $\kappa$ the specific conductivity of the suspension, $M$ the mass of particles per ml., $A_0$ their area per g., $u$ the velocity of sedimentation of the suspension in a given solution, and $u_0$ the limiting rate of settling, measured in concentrated electrolyte solutions. For spherical particles equation (1) simplifies to

$$\sigma^2 = \rho_2\eta\kappa(u_0 - u)/2Mu \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where $\eta$ is the viscosity of the solution. The electrokinetic potential $\zeta$ may then be calculated by using the equation

$$\sigma = \left(\frac{2n\varepsilon kT}{\pi}\right)^{\frac{1}{2}} \sinh\left(\frac{ze\zeta}{2kT}\right) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

where $\varepsilon$ is the dielectric constant in the diffuse part of the double layer (see Part I), $k$ is the Boltzman constant, $e$ the charge on the electron, $T$ the absolute temperature, and $n$ the number of charge-determining ions, of valency $z$, per ml. of solution. As pointed out in Part I, $n$ includes ions supplied by the water and also, in the case of glass, by ionisation of the surface. In the suspensions used, the contribution of such ions to the total conductivity was found to be consistently 4—5 gemmhos (see Hirschler, Thesis, London, 1951), the estimated contribution to $n$ being about $3 \times 10^{-5}$N. In our calculations this amount has

* Part I, *J.*, 1952, 286.

been added to the potassium chloride concentration to give the final value of $n$. In view of the fairly large value of this correction factor, values of $\zeta$ are only given for potassium chloride solutions of $10^{-4}$N and higher concentrations. In fact, it is found from the form of equation (3) that for a given surface charge, the calculated potential is not very sensitive to the value of $n$ taken, the 30% correction to $n$ applied for the $10^{-4}$N-solution leading to only a 3% correction in $\zeta$.

The figure shows the results obtained for $\sigma$, with two specimens of microspheres and one specimen of Pyrex powder, the agreement between the results obtained from these different batches being very good. The table gives the value of charge, taken from the graph, for a

| KCl, N | $2 \times 10^{-3}$ | $1 \times 10^{-3}$ | $5 \times 10^{-4}$ | $2 \times 10^{-4}$ | $1 \times 10^{-4}$ | $5 \times 10^{-5}$ | $2 \times 10^{-5}$ | $1 \times 10^{-5}$ | $5 \times 10^{-6}$ |
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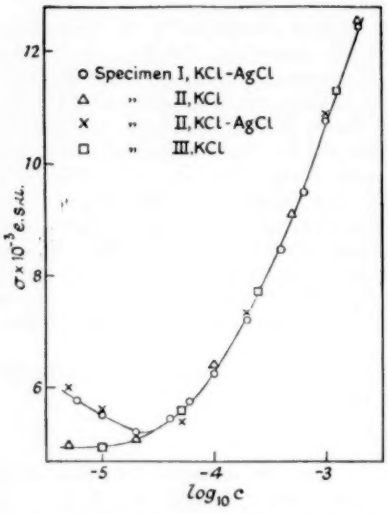


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## Experimental

*Materials.*—(i) Pyrex glass powder was prepared by grinding clean Pyrex glass in a mortar, followed by ball milling in an all-porcelain mill for several hours. Particles in the size range 2—5 microns were then separated by fractional sedimentation. They were cleaned by treat-

ment with several changes of 18% hydrochloric acid, then with boiling nitric acid and aqua regia, and finally washed many times with conductivity water. (All water used in this investigation was " equilibrium " conductivity water, of specific conductivity about 0·8 gemmho.) The milling process produced a small amount of very fine, near-colloidal porcelain dust, which was easily removed from the glass in the fractional sedimentation process. (ii) Pyrex microspheres were made by Bloomquist and Clark's method (*loc. cit.*), in which a fine dust of Pyrex particles is introduced into the air stream of a blowpipe, and blown through the flame. After formation, they were cleaned as described above. Particle sizing of powder and microspheres was carried out as described in Part I (see also Hirschler, Thesis, *loc. cit.*).

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Sedimentation velocities for the suspensions were determined as described by Elton (*Proc. Roy. Soc.*, 1949, *A*, **197**, 568), a thermostat at 25·00° ±0·01° being used. The mass of particles per ml. of suspension was determined in each case at the conclusion of the set of experiments by weighing on a microbalance. Limiting rates of settling ($u_0$) and mass per ml. ($M$) were: Specimen I (microspheres) $3{\cdot}46_9 \times 10^{-4}$ cm./sec., $5{\cdot}90 \times 10^{-4}$ g./ml.; Specimen II (microspheres) $3{\cdot}19_7 \times 10^{-4}$ cm./sec., $8{\cdot}22 \times 10^{-4}$ g./ml.; Specimen III (powder) $3{\cdot}80_5 \times 10^{-3}$ cm./sec., $4{\cdot}50 \times 10^{-4}$ g./ml.

Surface-area determination for Specimen III was done as described in Part I, the method of catalytic decomposition of hydrogen peroxide being used: $A_0 = 7{\cdot}81 \times 10^3$ cm.²/g.

BATTERSEA POLYTECHNIC, S.W.11. [*Received, March 5th*, 1952.]

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## 565. *Acid–Base Catalysis in Non-aqueous Solvents. Part XIII.* The Depolymerisation of Paraldehyde in Ethereal Solutions of Proton Acids and Lewis Acids.*

By R. P. BELL and B. G. SKINNER.

Kinetic measurements have been made on the depolymerisation of paraldehyde in ether at 0°, catalysed by hydrogen chloride, hydrogen bromide, and a number of metallic halides. The reaction is throughout of the second order with respect to the catalyst, and there are no essential differences between proton acids and Lewis acids, though the latter include the most effective catalysts. No co-catalyst is needed.

THE term *acid* is commonly taken to mean a species (molecule or ion) which has a tendency to lose a proton, but the same word is now sometimes used to denote molecules or ions which have a tendency to accept an electron-pair (*e.g.*, $BF_3$, $Ag^+$, etc.). Opinions differ as to whether it is desirable to use the word acid in this second sense (cf. Bell, *Quart. Reviews*, 1947, **1**, 113), but these questions of nomenclature are of little significance, and it is of much greater importance to obtain experimental evidence of the behaviour of the two classes of reagent. In this paper we shall refer to them as *proton acids* and *Lewis acids* respectively.

G. N. Lewis's extended use of the word acid was based partly on certain experimental similarities, including the power of both classes of acid to catalyse a variety of reactions. While this is true in a general sense, there are few instances in which catalysis by both proton acids and Lewis acids has been established for one and the same reaction, and no investigations in which a quantitative comparison has been made between the catalytic powers of acids of both classes. Quantitative measurements with Lewis acids are difficult to carry out, since the reaction velocity is often greatly affected by the presence of small quantities of impurities, which may act either as inhibitors or as co-catalysts. There are in fact a number of reactions in which Lewis acids exert a catalytic effect only when a small quantity of proton acid is present (cf., *e.g.*, Plesch, Polanyi, and Skinner, *J.*, 1947, 257; Plesch, *J.*, 1950, 543; Evans and Meadows, *J. Polymer Sci.*, 1949, **4**, 359; Norrish and Russell, *Trans. Faraday Soc.*, 1952, **48**, 91). In these circumstances the action of the

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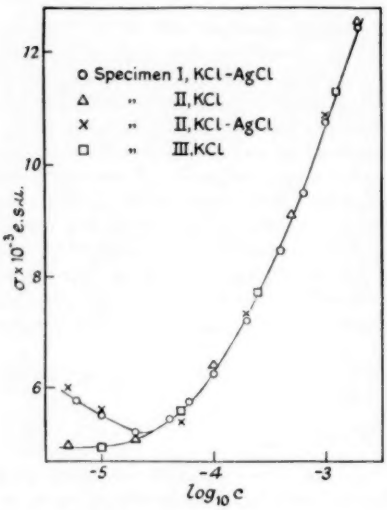


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THE term *acid* is commonly taken to mean a species (molecule or ion) which has a tendency to lose a proton, but the same word is now sometimes used to denote molecules or ions which have a tendency to accept an electron-pair (*e.g.*, $BF_3$, $Ag^+$, etc.). Opinions differ as to whether it is desirable to use the word acid in this second sense (cf. Bell, *Quart. Reviews*, 1947, **1**, 113), but these questions of nomenclature are of little significance, and it is of much greater importance to obtain experimental evidence of the behaviour of the two classes of reagent. In this paper we shall refer to them as *proton acids* and *Lewis acids* respectively.

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catalyst depends upon the transfer of a proton from the proton acid with the co-operation of the Lewis acid, *e.g.*, $BF_3 + H_2O + S \longrightarrow [BF_3OH]^- + SH^+$, where S is the substrate.

The depolymerisation of paraldehyde has proved to be a suitable reaction for comparing the catalytic effect of acids of both classes. It has previously been shown (Bell, Lidwell, and Vaughan-Jackson, *J.*, 1936, 1792) that the depolymerisation is catalysed by carboxylic acids and hydrogen chloride in anisole, benzene, and nitrobenzene solution, the reaction being strictly of the first order with respect to paraldehyde and approximately of the second order with respect to the catalyst (apparent order 1·6—2·5). Acid catalysis has also been demonstrated in the depolymerisation of paraldehyde vapour, taking place on the surface of the reaction vessel (Bell and Burnett, *Trans. Faraday Soc.*, 1937, **33**, 355). Preliminary tests showed that Lewis acids also were very effective catalysts in solution, though in anisole and chlorobenzene the reactions were too fast for quantitative study, being greatly inhibited by traces of moisture. Satisfactory results were, however, obtained by working at 0° in diethyl ether, where the basic properties of the solvent are sufficient to reduce the electrophilic powers of the Lewis acids by co-ordination.

## Experimental

*Preparation of Materials.*—The solvent used was carefully purified from alcohol and acetaldehyde, since the former was found to retard the reactions greatly and the latter is the product of depolymerisation. Commercial solvent ether was shaken with fifteen successive portions of water, dried ($CaCl_2$), and kept over sodium for at least 4 days. The ether was then distilled through a column, 8″ high, packed with pellets of sodium hydroxide. Whenever possible the solvent was distilled directly into the reaction vessel at this stage. For some experiments "AnalaR" ether was distilled through the sodium hydroxide column: the kinetic results were the same with both ether preparations. Paraldehyde was purified by fractional distillation, only the middle fraction being retained.

The catalysts were employed in the form of concentrated solutions in ether. "AnalaR" ferric chloride was twice sublimed in a current of dry air and dropped into dried ether, a slight cloud of oxide being removed by decantation. The concentration of the solution was determined by reduction with stannous chloride and titration with dichromate. Aluminium chloride solution was similarly prepared from the re-sublimed solid, and the solution analysed gravimetrically by precipitation with oxine. Ferric bromide could not be purified by sublimation, on account of decomposition, and commercial samples of this compound were found to have an unexpectedly low catalytic activity. Ethereal solutions were therefore prepared by the following method. Pure iron wire was allowed to react for a fortnight at room temperature with an excess of "AnalaR" bromine. The excess of bromine was removed by a current of dry air, and dry ether distilled into the same vessel. The solution was decanted from a small residue and analysed colorimetrically by means of the thiocyanate complex (cf. Allport, "Colorimetric Analysis," Chapman and Hall, p. 53). Stannic chloride (Kahlbaum) was redistilled in a current of dry air. Solutions more concentrated than about 0·04M could not be prepared because of the formation of a sparingly soluble addition compound with the ether. Their concentrations were estimated by hydrolysis and titration with sodium hydroxide. Titanium tetrachloride was redistilled twice in a current of dry air, and the ethereal solution analysed gravimetrically by precipitation of the titanium with oxine. Boron trichloride was redistilled twice, and the ethereal solution analysed by hydrolysis and titration with sodium hydroxide (methyl-red). Fused sticks of zinc chloride were scraped and dropped into dry ether. The solution was decanted from a small residue of zinc hydroxide, and its concentration determined gravimetrically by precipitation with oxine. Hydrogen bromide was prepared by dropping bromine on to a mixture of red phosphorus and water and was passed successively through moist red phosphorus, anhydrous calcium bromide, and phosphoric oxide before absorption in dry ether. Hydrogen chloride was prepared from sulphuric acid and sodium chloride and dried with calcium chloride and phosphoric oxide.

*Measurement of Reaction Velocity.*—The reactions were carried out in vessels of the type previously described (Bell and Levinge, *Proc. Roy. Soc.*, *A*, 1935, **151**, 211) immersed in melting ice. During any additions or withdrawals a current of air dried by passage through calcium chloride and solid sodium hydroxide was passed through the open space at the top of the reaction vessel. This precaution was necessary, since small quantities of moisture could greatly retard the reaction, or even stop it altogether, especially when very effective catalysts were being used

in low concentrations. In starting a reaction dry ether and catalyst solution were successively added to the weighed vessel, and the whole was brought to 0°, after which approx. 0·1 g. of paraldehyde was added from a calibrated pipette. Samples of about 0·7 g. were withdrawn at intervals and run into 25 c.c. of 0·1M-sodium hydrogen sulphite solution. The amount of acetaldehyde formed was then determined volumetrically by the method described by Bell, Lidwell, and Vaughan-Jackson (*loc. cit.*). Tests showed that none of the catalysts used interfered with the determination of acetaldehyde. In presence of stannic chloride fading end-points were at first obtained, but this was prevented by allowing the aldehyde–bisulphite mixture to stand for 5 minutes with the excess of iodine before adding sodium hydrogen carbonate. It is probable that some of the stannic chloride is reduced to the stannous condition by the bisulphite, and that the stannous ion is reoxidised only slowly by the iodine.

## Results

The reactions were always initially of the first order, and remained so until at least one-third of the paraldehyde had been converted into acetaldehyde. In the later stages of the reaction the velocity fell off more rapidly than was demanded by a first-order law, and with most catalysts the depolymerisation stopped when 60—70% of the theoretical amount of acetaldehyde had been formed. This value refers to an initial paraldehyde concentration of about 0·05M : the extent of conversion was greater with more dilute solutions, but it was not noticeably dependent upon the catalyst concentration. This suggests that the equilibrium conversion of paraldehyde into acetaldehyde is incomplete under our conditions, and this view is supported by the quantitative data given in the Table.

*Equilibrium in presence of* 0·03M-*aluminium chloride.*

| Initial [paraldehyde] | Depolymerisation, % | $K$ = [acetaldehyde]$^3$/[paraldehyde] |
|---|---|---|
| 0·0146 | 86·5 | 0·028 |
| 0·0160 | 85·1 | 0·029 |
| 0·0233 | 73·3 | 0·022 |
| 0·0270 | 76·3 | 0·037 |
| 0·0336 | 67·2 | 0·028 |
| 0·0356 | 66·0 | 0·029 |
| 0·0392 | 60·7 | 0·024 |
| 0·0482 | 60·4 | 0·035 |
| | | Mean $K$ = 0·029 |

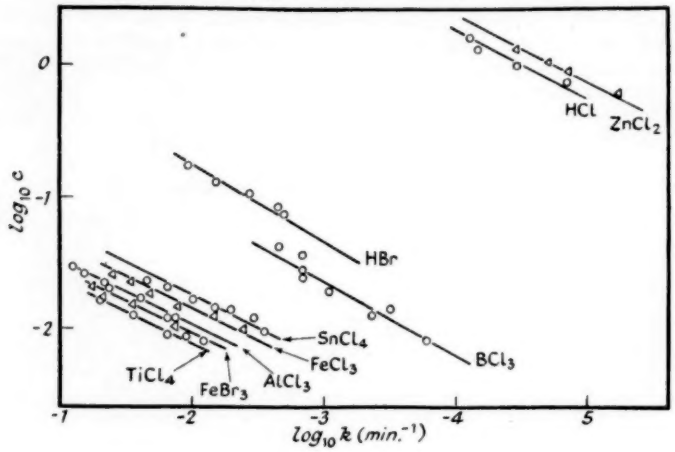
The constancy of $K$ is as good as could be expected, since in this range an error of 1% in measuring the percentage depolymerisation produces an error of 10% in $K$. Further support comes from some experiments with 0·02M-ferric chloride as catalyst in which equilibrium was approached from both ends in two separate experiments, giving $K = 0·026$ and 0·035 respectively. These equilibrium constants at 0° accord reasonably with the finding by Bell, Lidwell, and Vaughan-Jackson (*loc. cit.*) that a 1% solution in benzene at 20° depolymerises to the extent of 80% (corresponding to $K = 0·27$) : the polymerisation of the pure liquid is exothermic to the extent of 12·6 kcal. per mole of paraldehyde.

The setting up of an equilibrium does not give a complete explanation of the behaviour of the latter part of the reaction. If the reaction mixtures were kept for a long time the aldehyde concentration decreased again slowly, probably because of its conversion into another type of polymer, such as metaldehyde. Moreover, with hydrogen bromide and zinc chloride as catalysts (and probably also with boron trichloride and hydrogen chloride) the reaction did not stop at 60—70% conversion but went to 90% or further. These catalysts had to be used in high concentrations to obtain convenient reaction rates, and it is likely that they stabilise the acetaldehyde by co-ordination, thus displacing the equilibrium. It is clear, however, that none of these complications will affect the early part of the reaction, and the velocity constants in the Figure were obtained from a logarithmic plot of the first 30—50%; the first-order velocity constants are in units of $\log_{10}$ min.$^{-1}$ and the catalyst concentrations in moles/1000 g. With titanium tetrachloride the logarithmic plots fell off very steeply, and the constants given for this catalyst are obtained from initial slopes.

### Discussion

The data obtained (cf. Figure) are not of high accuracy or reproducibility, but are sufficient to establish a number of general points about the reaction. The Lewis acids appear to be able to act as catalysts without the co-operation of a proton acid, since none of the latter is present in the solution, and the addition of small quantities of water has a retarding effect. The experiments with hydrogen bromide and chloride show that no appreciable catalysis would be caused by small quantities of these substances formed by hydrolysis. The order of the reaction with respect to catalyst is always close to two, and the lines in the Figure are drawn with a slope of exactly two. Similar behaviour was found in previous work with proton acids in other solvents (Bell, Lidwell, and Vaughan-Jackson, *loc. cit.*). This second-order dependence on catalyst concentration suggests that the paraldehyde molecule has to be attacked by two catalyst molecules before depolymerisation takes place. Even in the absence of catalyst three acetaldehyde molecules can be formed by an electronic displacement, and the rôle of the acid catalyst will be to facilitate the formation of the

*Depolymerisation of paraldehyde in ether* (c = *catalyst concn.*)




carbon–oxygen double bond by its electrophilic action. If two carbonyl groups are formed this automatically leads to the formation of the third and the break-down of the whole molecule, and it is therefore reasonable that two catalyst molecules should be required. It may be noted that the removal of only one acetaldehyde molecule from the paraldehyde ring leaves behind an unstable species (either a di-radical or the ion $\bar{O}\cdot CHMe\cdot O\cdot \overset{+}{C}HMe$), and it is more probable that the depolymerisation takes place in a single step.

The Figure shows that even a very strong proton acid such as hydrogen bromide is inferior as a catalyst to almost all the Lewis acids. The latter lie fairly close together in their catalytic effects, with the exception of zinc chloride, which is a very weak catalyst. There is already ample evidence that the relative catalytic effects of Lewis acids vary considerably from one reaction to another, even the order of effectiveness being frequently changed (cf., *e.g.*, Dermer and Billmeier, *J. Amer. Chem. Soc.*, 1942, **64**, 465). This behaviour is probably connected with steric effects, and appears in equilibria as well as in kinetics, thus making it impossible to arrange Lewis acids in any unique order of strengths (Lewis, *J. Franklin Inst.*, 1938, **226**, 293).

The Figure shows that hydrogen bromide is about 100 times as efficient a catalyst as hydrogen chloride, the catalytic efficiency being taken as inversely proportional to the concentration needed to produce a given velocity. Similarly, Bell and Burnett (*Trans. Faraday Soc.*, 1939, **35**, 324) found hydrogen bromide to be about 300 times as effective as hydrogen chloride in the depolymerisation of trioxymethylene in decalin solution. It is

generally agreed that hydrogen bromide is the stronger acid of the two, but there is little quantitative information as to their acid strengths. Kolthoff (*J. Amer. Chem. Soc.*, 1934, **56**, 1007) measured the conductivities of 0·005M-solutions in glacial acetic acid and found these to be in the ratio HCl : HBr = 9 : 160, indicating a ratio of dissociation constants of about 1 : 300. Calculation from thermodynamic data shows that in the gas phase the dissociation $HX \longrightarrow H^+ + X^-$ requires about 10 kcal./mole more for HCl than for HBr (Bell and Burnett, *loc. cit.*, 1939), but the difference will be less in solution, since the solvation energy of $Cl^-$ will be greater than that of $Br^-$.

Our thanks are offered to the Department of Scientific and Industrial Research for a maintenance grant to one of (B. G. S.).

PHYSICAL CHEMISTRY LABORATORY, OXFORD. [*Received, March 17th*, 1952.]

## 566. *Carbohydrate Derivatives of Alkyl Dithiocarbazinates.*

By R. HULL.

A new series of sugar derivatives is obtained from dithiocarbalkoxyhydrazines (alkyl dithiocarbazinates) (I). The structures of abnormal products obtained from D-glucosamine and D-fructose are discussed.

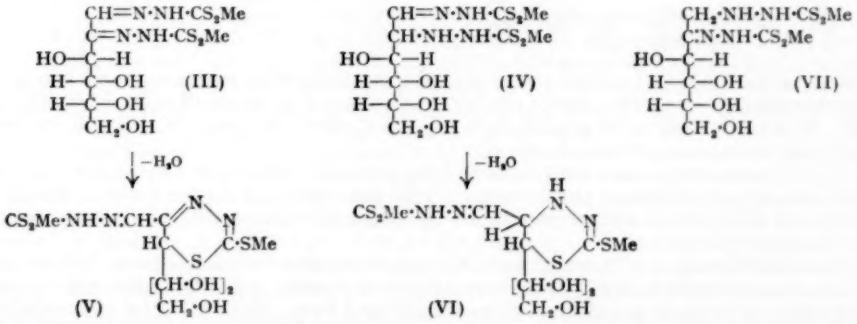
INTERACTION of dithiocarbomethoxyhydrazine (methyl dithiocarbazinate) (I; R = Me) and aldehydes has been shown to yield derivatives of type (II) (Busch, *J. pr. Chem.*, 1916, **93**, 60). A series of sugar derivatives has now been prepared by using various aldoses and

(I) $NH_2{\cdot}NH{\cdot}CS{\cdot}SR$ $R'{\cdot}CH{=}N{\cdot}NH{\cdot}CS{\cdot}SR$ (II)

dithiocarbalkoxyhydrazines in aqueous-alcoholic solution. The methyl ester (I; R = Me) gave, in the majority of cases, crystalline products, which would serve as useful derivatives for the identification of the respective sugar. The ethyl ester (I; R = Et) usually gave oils, some of which slowly crystallised, whereas the products from the *iso*propyl ester (I; R = $Pr^i$) remained oils.

It appeared that the derivatives have open-chain structures since, in the one case investigated, *N*-dithiocarbomethoxy-*N'*-D-*gluco*pentahydroxyhexylidenehydrazine (II; R = Me, R' = $C_5H_{11}O_5$), the absorption spectrum had maxima and minima similar to those of the ethylidene compound (II; R = R' = Me) and showed no mutarotation.

Similar derivatives have been prepared from D-galactose, L-arabinose, and lactose, and, with the ethyl ester, from D-glucose and D-galactose.



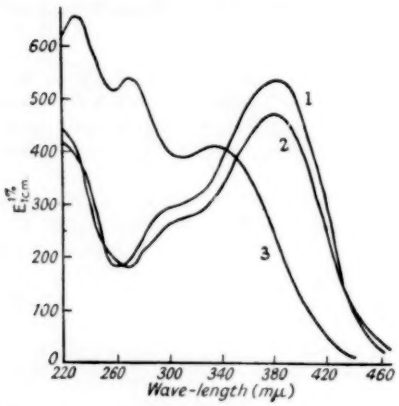
Reaction of dithiocarbomethoxyhydrazine with D-glucosamine was abnormal in that two molecular proportions of ester reacted with the sugar. From the analysis the reaction product could be (III) or (IV), the former being obtained by an osazone type of mechanism and (IV) by reaction of a second molecule of the ester with the sugar with elimination of ammonia. The reaction was further complicated in that another molecule of water was

eliminated on crystallisation; this could give rise to (V) or (VI) or an anhydro-compound in which water was eliminated from the sugar chain. If (III) was formed by an osazone-type of mechanism, it would be expected that D-glucose and an excess of dithiocarbomethoxyhydrazine would give the same compound: this did not appear to be the case, for only the one condensation product (II; R = Me, R′ = $C_5H_{11}O_5$) was obtained when more than three mols. of ester were used. However, with D-fructose a bis-condensation product was obtained which gave an analysis similar to that of (III or IV) and absorption bands similar to those of (V or VI) but differed markedly in its decomposition point from the D-glucosamine condensation product. It seemed that neither the D-fructose nor the D-glucosamine condensation product contained ethylenic linkages to both $C_{(1)}$ and $C_{(2)}$ of the sugar chain because their absorption bands, which were almost identical (see Figure), differed from that of diacetyl bisdithiocarbomethoxyhydrazone, [·CMe:N·NH·$CS_2$Me]$_2$. It is suggested therefore that the glucosamine and the fructose condensation product are respectively (IV) [giving (VI) on crystallisation] and (VII).

Two molecular proportions of dithiocarbethoxyhydrazine also reacted with one of D-glucosamine, but the product, which was not further investigated, did not lose water on crystallisation.

## Experimental

*N-Dithiocarbomethoxy-N′-D-glucopentahydroxyhexylidenehydrazine* (II; R = Me, R′ = $C_5H_{11}O_5$).—(*a*) A mixture of D-glucose (5·1 g.) and dithiocarbomethoxyhydrazine (methyl dithiocarbazinate) (3·4 g.) was heated under reflux in methanol (11·3 c.c.) and water (11·3 c.c.) during 20 minutes. Next morning the solid (7·7 g.) was collected and washed with aqueous




1, D-*Glucosamine condensation product in* N/1000-NaOH.
2, D-*Fructose condensation product in* N/2000-NaOH.
3, *Diacetyl bisdithiocarbomethoxyhydrazone in* N/1000-NaOH.

alcohol; it had m. p. 117—119°. The *product* crystallised from methanol–water (95 : 5) in colourless needles, m. p. 120—121°, $[\alpha]_D^{20}$ +23·2° (*c*, 4·088 in pyridine) (Found: C, 31·65; H, 5·95; N, 9·0; S, 21·5. $C_8H_{16}O_5N_2S_2$,$H_2O$ requires C, 31·8; H, 5·95; N, 9·25; S, 21·2%). Light absorption: $\gamma_{max.}$ 275 mμ (ε, 9541).

(*b*) D-Glucose (0·9 g.) and dithiocarbomethoxyhydrazine (1·95 g.) in alcohol (2·5 c.c.) and water (3·0 c.c.) were warmed gently during ½ hour and then set aside for 2 days. The solid (1·5 g.) was collected and after crystallisation from alcohol had m. p. 120—121° alone or mixed with the product from (*a*).

*N-Dithiocarbomethoxy-N′-L-arabotetrahydroxyamylidenehydrazine.*—L-Arabinose (2·9 g.) and dithiocarbomethoxyhydrazine (2·37 g.) were heated under gentle reflux in alcohol (5·5 c.c.) and water (5·5 c.c.) during 20 minutes and then set aside for 2 days. The solid (4·0 g.) was collected and washed with aqueous alcohol. Crystallisation from aqueous methanol gave the *compound* in colourless needles, m. p. 163—164° (decomp.), $[\alpha]_D^{20}$ +15·3° (*c*, 3·006 in pyridine) (Found: C, 33·5; H, 5·95; N, 11·5: $C_7H_{14}O_4N_2S_2$ requires C, 33·1; H, 5·5; N, 11·05%).

*N-Dithiocarbomethoxy-N′-D-galactopentahydroxyhexylidenehydrazine.*—D-Galactose (3·6 g.) and the methyl ester (2·44 g.) in water (8·0 c.c.) and alcohol (7·0 c.c.) gave, as above, the *compound* as colourless needles (from alcohol), m. p. 105°, $[\alpha]_D^{22}$ +16·4° (*c*, 3·016 in pyridine)

(Found: C, 32·95; H, 5·8; N, 9·5; S, 21·6. $C_8H_{16}O_5N_2S_2,0{\cdot}5H_2O$ requires C, 32·8; H, 5·8; N, 9·55; S, 21·8%).

*N-Dithiocarbomethoxy-N'-(4-β-galactosyloxy-D-glucotetrahydroxyhexylidene)hydrazine.*—Lactose (5·0 g.) and the methyl ester (1·8 g.) in methanol (6 c.c.) and water (12 c.c.) gave, as above, the *product* in colourless needles (from 1 : 1 aqueous methanol), m. p. 165—166° (decomp.) (placed in copper block at 160°), $[\alpha]_D^{22}$ +18·9° (*c*, 3·785 in pyridine) (Found: C, 37·35; H, 5·8; N, 6·3; S, 14·6. $C_{14}H_{26}O_{10}N_2S_2$ requires C, 37·65; H, 5·8; N, 6·3; S, 14·35%).

*N-Dithiocarbomethoxy-N'-ethylidenehydrazine.*—Acetaldehyde (1·8 g.) and the methyl ester (4·99 g.) in ethanol (15 c.c.) and water (5 c.c.) were warmed at 40—50° overnight. The solid (1·8 g.) was collected and washed with alcohol. Crystallisation from alcohol (95%) gave colourless needles of *N-dithiocarbomethoxy-N'-ethylidenehydrazine*, m. p. 166—167° (Found: C, 32·35; H, 5·5; N, 18·7. $C_4H_8N_2S_2$ requires C, 32·4; H, 5·4; N, 18·9%).

*Reaction of D-Glucosamine with Dithiocarbomethoxyhydrazine.*—D-Glucosamine hydrochloride (6·47 g.) and crystalline sodium acetate (4·08 g.) in water (24 c.c.) were treated with dithiocarbomethoxyhydrazine (7·35 g.) in alcohol (24 c.c.), heated 85° ± 5° during 1 hour, and then kept for 2 days. The *product* (? IV) (3·65 g.), collected and washed with alcohol, had m. p. 187° (decomp.) (Found: C, 31·4; H, 4·7; N, 14·9; S, 32·8. $C_{10}H_{20}O_4N_4S_4$ requires C, 30·95; H, 5·15; N, 14·4; S, 33·0. $C_{10}H_{18}O_4N_4S_4$ requires C, 31·1; H, 4·7; N, 14·5; S, 33·1%). Crystallisation from aqueous 2-ethoxyethanol gave the *tetrahydrothiadiazine* (? VI) as yellow needles, m. p. 198° (decomp.), $[\alpha]_D^{24}$ +417° (*c*, 3·022 in pyridine) (Found: C, 32·4; H, 4·8; N, 14·9; S, 33·9. $C_{10}H_{18}O_2N_4S_4$ requires C, 32·4; H, 4·85; N, 15·15; S, 34·6%).

*Reaction of D-Fructose with Dithiocarbomethoxyhydrazine.*—D-Fructose (0·9 g.) and the methyl ester (1·22 g.) were heated under gentle reflux in alcohol (5 c.c.) and water (1·5 c.c.) during 1 hour. Next morning the *product* (? VII) (0·5 g.) was collected and washed with alcohol. Crystallisation from aqueous methanol gave orange-yellow needles, m. p. 164° (decomp.), $[\alpha]_D^{24}$ +208·4° (*c*, 3·022 in pyridine) (Found: C, 30·4; H, 4·9; N, 14·1. $C_{10}H_{18}O_4N_4S_4,0{\cdot}5H_2O$ requires C, 30·4; H, 4·8; N, 14·15%).

*Diacetyl Bisdithiocarbomethoxyhydrazone.*—Diacetyl (3·1 g.) and the methyl ester (8·8 g.) were heated in methanol (55 c.c.) and water (15 c.c.) during ½ hour. After a further 3 hours the solid (10·5 g.) was collected and washed with alcohol. The *compound* crystallised from hot dimethylformamide on slow addition of water, in light yellow prismatic needles, m. p. 228—229° (decomp.) (Found: C, 32·6; H, 4·35; N, 18·9. $C_8H_{14}N_4S_4$ requires C, 32·65; H, 4·75; N, 19·05%).

*Dithiocarbethoxyhydrazine* (I; R = Et).—To potassium hydroxide (22·4 g.) in absolute alcohol (150 c.c.), hydrazine hydrate (70%; 28 c.c.) was added, followed slowly by distilled carbon disulphide (24·4 c.c.), the temperature being kept below 20°. The solid potassium salt was collected, washed with alcohol, and after dissolution in water (200 c.c.) and alcohol (30 c.c.) treated, with shaking, with ethyl bromide (33·8 c.c.) for 2 hours. The oil was extracted with ether and dried, and the solvent removed. The semi-solid material was used as such in the subsequent condensations.

*N-Dithiocarbethoxy-N'-D-glucopentahydroxyhexylidenehydrazine* (II; R = Et, R' = $C_5H_{11}O_5$). —A mixture of D-glucose (5·15 g.) and the ethyl ester (3·9 g.) was heated under reflux during 10 minutes in alcohol (3 c.c.) and water (3 c.c.), needles contaminated with oily products being formed on cooling. Crystallisation from aqueous alcohol gave the *product* as colourless silky needles (2·7 g.), m. p. 89·5—90·5°, $[\alpha]_D^{20}$ +18·95° (*c*, 3·59 in pyridine) (Found: C, 34·95; H, 6·7; N, 8·75; S, 20·65. $C_9H_{18}O_5N_2S_2,0{\cdot}75H_2O$ requires C, 34·6; H, 6·3; N, 9·0; S, 20·55%).

*N-Dithiocarbethoxy-N'-D-galactopentahydroxyhexylidenehydrazine.*—D-Galactose (2·5 g.) and the ethyl ester (1·9 g.) were heated under gentle reflux during 10 minutes in alcohol (1·5 c.c.) and water (1·5 c.c.). After a long time the *product* (0·8 g.) was collected and crystallised from aqueous alcohol in almost colourless prismatic needles, m. p. 141—142°, $[\alpha]_D^{20}$ +13·8° (*c*, 3·33 in pyridine) (Found: C, 36·4; H, 5·95; N, 9·45. $C_9H_{18}O_5N_2S_2$ requires C, 36·25; H, 6·05; N, 9·4%).

*Reaction of Glucosamine with Dithiocarbethoxyhydrazine.*—Glucosamine hydrochloride (1·58 g.) and crystalline sodium acetate (1·0 g.) in water (6 c.c.) were treated with a solution of the ethyl ester (2·0 g.) in alcohol (6 c.c.) and heated at 80° ± 5° during ½ hour. On cooling, the yellow *product* (0·7 g.) was collected and washed with aqueous alcohol. Crystallisation from aqueous alcohol gave yellow prismatic needles, m. p. 145—146° (decomp.) (Found: C, 34·7; H, 5·5; N, 13·3. $C_{12}H_{24}O_4N_4S_4$ requires C, 34·6; H, 5·75; N, 13·45%).

*Dithiocarboisopropoxyhydrazine.*—Potassium hydroxide (22·4 g.) in absolute alcohol (80 c.c.) was treated with hydrazine hydrate (70%; 28 c.c.), with stirring. Distilled carbon disulphide (24·4 c.c.) was added during ¾ hour, the temperature being kept below 20°. After 3·5 hours'

stirring, the solid potassium salt was collected, washed with alcohol, and dissolved in water (200 c.c.). *iso*Propyl bromide (41 c.c.) was added and the mixture shaken overnight. The oily *ester* was extracted in ether and dried, and the ether removed under diminished pressure. The solid (17·8 g.; m. p. 69—71°) remaining crystallised from benzene in colourless prisms, m. p. 79—80° (Found: N, 18·65; S, 42·8. $C_4H_{10}N_2S_2$ requires N, 18·65; S, 42·7%).

IMPERIAL CHEMICAL INDUSTRIES LIMITED,
RESEARCH LABORATORIES, BLACKLEY, MANCHESTER, 9. [*Received, April 7th*, 1952.]

## 567. *The Metal-catalysed Reaction between Acetylene and Hydrogen. Part VI.* The Reaction over Rhodium and Iridium.*

By J. SHERIDAN and W. D. REID.

The reaction between acetylene and hydrogen proceeds over rhodium and iridium very similarly to that over other group-VIII metals. In the reaction over rhodium, which is a fairly active catalyst, the yields of $C_2$ hydrocarbons (ethylene, ethane) are 70—80% of the acetylene removed, the rest of the removed acetylene appearing as partially hydrogenated polymers. The $C_2$ hydrocarbon yield is somewhat greater at large hydrogen : acetylene ratios, but the dependence on this ratio is very slight. The orders of reaction (first with respect to hydrogen, zero with respect to acetylene) therefore apply to both the simple hydrogenation and the hydropolymerisation. The effect of temperature on the total initial rate corresponds to an apparent activation energy of 15·5 kcals. Iridium is rather weakly active, and produces largely simple hydrogenation, with a small amount of hydropolymerisation. The orders of reaction over iridium are similar to those over rhodium. Ruthenium and osmium are only very slightly active as catalysts. The catalytic effects of all the metals of group VIII are briefly discussed and compared.

IN recent years importance has been attached to comparisons of the kinetics of a given catalytic process over series of related catalysts. The acetylene–hydrogen reaction has been investigated over a variety of metals, and, with nickel, palladium, platinum, and iron, some details of its behaviour have been elucidated (Parts I—V, *J.*, 1944, 373; 1945, 133, 301, 305, 470). Although in this reaction there are two stages of hydrogenation, and also production, to varying extents, of reduced polymers of acetylene, these complexities make possible the ready detection of differences in the behaviour of different metals as catalysts. Apart from possessing clear differences in activity, metals differ in the relative extents to which they produce polymerisation and hydrogenation, and in the extents to which the latter is selective in yielding ethylene rather than ethane while any appreciable amount of acetylene remains. These variations are superimposed on a significant similarity in kinetic behaviour over all catalysts studied, which indicates always a very strong adsorption of acetylene, this being stronger than the adsorption of ethylene on the same catalyst. Variations in activation energies with different metals are observed, but are not very marked.

In view of the results reported for the hydrogenation of ethylene, and in particular of the great activity of rhodium in this reaction (Beeck, *Rev. Mod. Physics*, 1945, **17**, 61), it was felt desirable to extend work on the acetylene–hydrogen reaction to the use of those group-VIII metals not previously studied. This would make possible a wider comparison between the actions of various metals and assist the ultimate understanding of their actions in terms of their electronic and crystal structures. In the present paper, therefore, results are reported for rhodium, iridium, ruthenium, and osmium catalysts. Since this work was completed (1949) we have learnt of experiments by Beeck and Wheeler (cf. Beeck, *Discuss. Faraday Soc.*, 1950, **8**, 122) on the hydrogenation of acetylene over rhodium, as well as over other catalysts previously studied. Details of their results, however, have not yet been given.

* Part V, *J.*, 1945, 470.

## Experimental

*Apparatus.*—This was essentially as described in Part I.

*Gases.*—Cylinder acetylene was purified by passage through the reagents described by Conn, Kistiakowsky, and Smith (*J. Amer. Chem. Soc.*, 1939, **61**, 1868), but was not dried other than by passage over anhydrous calcium chloride. Cylinder hydrogen was freed from oxygen by passage through alkaline sodium hydrosulphite (dithionite) solution.

*Catalysts.*—All catalysts were carried on well cleaned pumice granules, and had metal : pumice ratios of 1 : 20. Ruthenium, rhodium, and iridium catalysts were made by evaporating solutions of ruthenium trichloride, rhodium trichloride, and ammonium hexachloroiridate on to the pumice with continuous stirring, and then drying them at 120°. Required amounts of ruthenium and rhodium catalysts were finally reduced with hydrogen in the reaction vessel at 240—250° for at least 2 hours (reduction was then complete). The ammonium hexachloroiridate was first decomposed at 120° in a stream of hydrogen, ammonium and hydrogen chlorides being evolved, and the residue finally reduced in hydrogen at 240° as before. Osmium catalysts were made by reduction of aqueous osmium tetroxide with alkaline formaldehyde in the presence of pumice. Water-soluble compounds were removed by washing the product, and the residual water was then evaporated, with stirring. The catalyst was heated in hydrogen to 150° immediately before use.

Approximately 2 g. of catalyst, including carrier, were used in each experiment.

*Analysis of Products.*—Gaseous products were analysed in a Bone–Newitt apparatus, the methods described in Part I being used.

## Results

*Ruthenium.*—Prepared as described, ruthenium had an extremely small activity as a catalyst. A slow reaction occurred at 288° but did not reach a rate convenient for study below temperatures at which the ordinary thermal reactions of acetylene in glass are known to begin. Therefore, though a definite slight catalytic effect is probable, no detailed study could be made.

*Osmium.*—Though slightly more active than ruthenium, osmium also had very little catalytic effect. With 400 mm. of hydrogen and 200 mm. of acetylene a pressure fall of 46 mm. occurred in 2 hours at 210°. Though this change is definitely faster than any reaction in the absence of osmium at this temperature, a convenient study could again not be made except at such temperatures that non-catalysed thermal reactions were also likely. Analysis, after a relatively small pressure fall, indicated that reaction was largely polymerisation, though accompanied by some hydrogenation.

*Rhodium.*—This metal was quite an active catalyst, and reaction over it could be conveniently studied at 50—100°. The fall in pressure with time followed courses similar to those obtained with nickel catalysts (Part I).

Some typical examples of analyses of products are given in the table ($\Delta p$ = fall in pressure in time $t$; $C_mH_n$ represents olefins higher than $C_2$ detected in the gas). With such initial mix-

| | Initial press. (mm.) | | | | Press. in product (mm.) | | | | | Yield (as % of $C_2H_2$ removed) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $T$ ° | $C_2H_2$ | $H_2$ | $\Delta p$ (mm.) | $t$ (mins.) | $C_2H_2$ | $C_mH_n$ | $C_2H_4$ | $H_2$ | $C_2H_6$ | $C_2H_4$ | $C_2H_6$ |
| 84·5 | 196 | 223 | 187 | 60 | 60 | 15 | 84 | 54 | 16 | 61 | 13 |
| 84·5 | 400 | 169 | 169 | 130 | 266 | 15 | 85 | 22 | 12 | 63 | 9 |
| 84 | 101 | 203 | 100 | 17 | 28 | 8 | 40 | 116 | 12 | 55 | 16 |
| 88 | 201 | 404 | 201 | 18 | 62 | 19 | 56 | 228 | 40 | 40 | 29 |

tures, some 70% of the acetylene used undergoes hydrogenation, giving ethylene with fair selectivity, and the remaining 30% is converted, with about an equal amount of hydrogen, into reduced polymers of acetylene. Since the pressures of higher olefins detected in the gas phase are about 40% of the pressures of acetylene removed as reduced polymers, these must be largely low polymers, *e.g.*, of the $C_4$ and $C_6$ series.

Variations in initial hydrogen : acetylene ratio affect the yields in the manner shown in Fig. 1. In these experiments the larger of the initial partial pressures was near 400 mm. A sixteenfold increase in initial hydrogen : acetylene ratio causes an increase in the yield of $C_2$ hydrocarbons from about 70% to about 85% of the removed acetylene. This relatively small change for such a wide change in ratio must mean that the orders of reaction for the hydrogenation are very similar to those for the polymerisation. Production of ethane, at the stages at which analyses were made (where $\Delta p$ = initial pressure of the less rich component),

was small when acetylene was in a large excess, but increased about fourfold for a sixteenfold increase in hydrogen : acetylene ratio, while the yield of ethylene simultaneously fell slightly.

The orders of reaction were studied by the method of initial rates (Fig. 2); the reaction is of first order with respect to hydrogen and of zero order (or somewhat lower) with respect to acetylene. Hence the kinetics of the reactions over rhodium closely resemble those over all other metals studied. Strong adsorption of acetylene is again indicated, and it seems very probable that the mechanism of reaction is essentially similar to that postulated for other catalysts in previous papers in this series.

The effect of temperature on the initial rate of pressure-fall was studied between 17° and 110°. A good straight line is obtained for the plot of the logarithm of the initial rate against the reciprocal of the temperature, the slope corresponding to an apparent activation energy of 15·5 ± 0·5 kcal.

*Iridium.*—This metal had a smaller but distinct activity, and could be used conveniently at about 175°. The pressure–time curves obtained were again similar to those for nickel catalysis.

The effects of variation of partial pressures on the initial rate were systematically studied; the results were very similar to those found for rhodium. Therefore the reaction is of first order

FIG. 1. *Effect of initial hydrogen : acetylene ratio on yields over rhodium at 84·5°.*

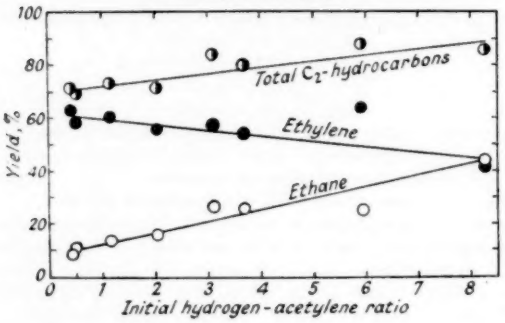
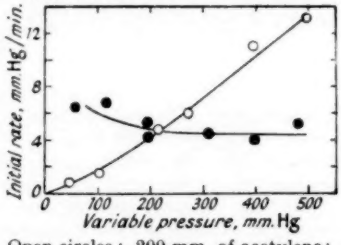



FIG. 2. *Effect of variation of partial pressure on the initial rate over rhodium at 85·5°.*




Open circles : 200 mm. of acetylene; hydrogen pressure varied.
Filled circles : 200 mm. of hydrogen; acetylene pressure varied.

with respect to hydrogen and of zero order with respect to acetylene, in resemblance to that over other metals.

Iridium catalysts were readily deactivated, and the products were analysed only for the reaction over partially deactivated catalysts. While considerable unchanged acetylene still remained, about 25% of the used acetylene appeared as ethylene and about 60% of it as ethane, the remaining 15% being removed (with an approximately equal amount of hydrogen) as reduced polymers. The high yield of ethane indicates a poor selectivity of hydrogenation, which is unexpected in view of the strong adsorption of acetylene indicated by the reaction orders. The effect may, however, be connected with the deactivated state of the catalyst, since a similar ethane predominance was also observed over highly deactivated palladium (Part V).

## DISCUSSION

Since experiments have now been made on all the metals of group VIII, and also copper, as catalysts for the acetylene–hydrogen reaction, it is convenient to discuss the results of the current experiments as part of a brief review of the differences in catalytic action shown by different metals.

*Activity of Catalysts.*—Considering catalysts in the most active forms which we have used, palladium is the most active, weight for weight, followed by platinum and then by nickel and rhodium, which are about equally active. Iron, copper, cobalt, and iridium are weakly active, and ruthenium and osmium almost inactive. In the absence of data on the specific surfaces of our catalysts such results must be very approximate, but there is good qualitative agreement with Beeck and Wheeler's results (Beeck, *loc. cit.*) for platinum, palladium, nickel, rhodium, and iron catalysts, in which surface areas are allowed for. New copper catalysts have quite definite activity, comparable with that of iron.

All the appreciably active metals are of face-centred cubic structure (nickel, palladium,

platinum, rhodium, iridium, and copper), while the least active (osmium and ruthenium) are of close-packed hexagonal structure. As cobalt may contain crystals of both these types (Hofer and Peebles, *J. Amer. Chem. Soc.*, 1947, **69**, 2497), it is not impossible that such activity as it shows is due to the cubic form, the close-packed hexagonal form being again inactive. Iron, with a body-centred cubic lattice, has a definite though small activity.

*Reaction Orders and Energies of Activation.*—In all cases where orders could be measured (nickel, palladium, platinum, iron, rhodium, and iridium) a remarkable consistency is found, indicative of very strong acetylene adsorption. In these circumstances the apparent activation energies, which would not require correction for the heat of adsorption of acetylene, should reflect any deep-seated differences in reaction mechanism, and they are found to be all rather similar in the cases measured. For nickel, platinum, palladium, iron, and rhodium the mean activation energies for the overall reaction are in the range 12—16 kcals.; only for nickel was it possible to estimate separate activation energies for hydrogenation and hydropolymerisation, but it seems probable that in general the activation energies are all close to the mean values. These facts all support a close general similarity in the mechanism of the reactions over these metals.

Beeck and Wheeler (*loc. cit.*) also find a constant energy of activation of the hydrogenation for nickel, rhodium, palladium, and platinum, but their figure (6—7 kcals.) is much lower than ours. This discrepancy, though presumably due to differences between our catalysts and Beeck and Wheeler's evaporated films, is somewhat puzzling.

*Extent of Hydropolymerisation.*—At comparable temperatures and gas pressures, this is greatest in the first horizontal row of metals—iron, cobalt, nickel, and copper. Copper readily produces a peculiarly specific polymerisation, relatively little hydrogen entering into the polymers. Of this set of metals, iron causes least polymerisation. In the second row of group VIII, rhodium and palladium are alike in producing considerably less polymer, but this still normally constitutes some 20—30% of the removed acetylene. With iridium and platinum (the third row of the group) the polymer yield has fallen to less than 20%. Polymerisation, therefore, seems chiefly associated with metals of smaller atomic radii. The situation was discussed in Part III.

*Selectivity of Hydrogenation to Ethylene.*—This can be assessed by comparison of the ethane : ethylene ratios, in the early stages of reaction, with comparable acetylene : hydrogen ratios. It is greatest with palladium, somewhat lower with nickel, and lower still with rhodium, platinum, and probably iridium. Over cobalt, selectivity is quite high, and with iron it shows variations with different samples of catalyst (Part V).

It is clearly not possible at present to give a full interpretation of comparisons of the above types in terms of the electronic and crystal structures of the metals concerned, but the following points may be noted.

Activity is mainly associated with face-centred cubic metals, close-packed hexagonal structures being scarcely active. This is possibly related to differences in interatomic distances in certain crystal faces. In particular, the longer spacings in the (110) faces of nickel, palladium, platinum, rhodium, and iridium are in the range 3·5—3·9 Å, and would all (as was shown for nickel, palladium, and platinum in Part I) be stereochemically suitable for associative chemisorption of acetylene, whereas in hexagonal cobalt, ruthenium, and osmium the $(10\bar{1}0)$ planes, which resemble the (110) case most closely, have longer metal–metal distances (4·11, 4·27, and 4·31 Å), which are probably too long to be spanned by adsorbed acetylene without considerable strain.

Maximum activity in each horizontal row of group VIII occurs in the column nickel, palladium, platinum, and this is parallel to a similar maximum in paramagnetic susceptibility in this column (cf. Maxted, *J.*, 1949, 1990). Palladium, with the highest susceptibility per gram-atom of those listed by Maxted, is in fact the most active catalyst in the present reaction, and the sequence of activities resembles the sequence of susceptibilities. Palladium is also the catalyst producing the most selective hydrogenation of acetylene. A parallel with magnetic properties in this case, however, may well be fortuitous, since the factors affecting selectivity must involve complex adsorption relationships, and it is certainly not valid to regard selectivity as a measure of the relative activities of a catalyst for the hydrogenation of acetylene and of ethylene on the same surface, since acetylene is always

able to displace ethylene strongly. In general, however, the parallel between activity and magnetic susceptibility reflects an undoubted influence of the electronic structure of the metal. The clear differences, electronically, between metals of the copper group and those of group VIII are also reflected in the specific polymerizing action of copper in the acetylene–hydrogen reaction (cf. Reynolds, *J.*, 1950, 266).

For the hydrogenation of ethylene a relationship has been proposed (Beeck, *loc. cit.*, 1950) between the activity of the catalyst and the percentage of *d*-character in the metallic bonding of the metal, as estimated by Pauling (*Proc. Roy. Soc.*, 1949, *A*, **196**, 343). Since the percentage of *d*-character reaches maxima at ruthenium and osmium, any influence purely of *d*-character ought to show parallel maxima. Actually these metals have virtually no activity for acetylene hydrogenation; in the hydrogenation of ethylene, where activity is thought to increase with *d*-character, they might be expected to be very active, but have not been tested. There appears to be no clear influence purely of *d*-character detectable on any characteristic of the acetylene–hydrogen reaction.

It might, however, be expected that any influence of *d*-character would be clearer within cases of a definite crystal type, such as the face-centred cubic metals. This seems to be so in the hydrogenation of ethylene, where activity in nickel, platinum, palladium, and rhodium increases steadily in that order, which is the order of increasing *d*-character. For the acetylene-hydrogen reaction, on the other hand, activity and selectivity, plotted against *d*-character, reach maxima at palladium, since iridium and rhodium, both with more *d*-character than palladium, are less active and less selective than that metal. Iridium, in particular, probably falls out of any smooth sequence, and it would be interesting to know if iridium has the high activity for the hydrogenation of ethylene which is predicted for a face-centred cubic metal with bonds containing nearly as much *d*-character as those in rhodium. Until data are obtained on a wider variety of metals than hitherto, it would seem wise to interpret with caution relationships between *d*-character and catalytic action, particularly where different types of crystal lattice are under consideration.

THE UNIVERSITY, BIRMINGHAM, 15. [*Received, February 18th*, 1952.]

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## 568. *The Effect of Water on the Decomposition of Benzoyl Peroxide.*

By C. F. H. TIPPER.

The effect of water on the decomposition of benzoyl peroxide between 70° and 100° in four solvents has been investigated. When phenol is used, the water acts as an inert diluent, but in dry or aqueous benzyl alcohol, acetic acid, or pyridine the reaction appears to be complex, the results being difficult to interpret. In dry acetic acid and pyridine the decomposition is retarded by oxygen, owing probably to the formation of a secondary reaction product, which reduces the induced chain decomposition. Addition of water increases the rate in these solvents considerably. However, the effect of water on the yield of carbon dioxide and non-volatile acid formed, and on the efficiency of the peroxide as a polymerisation catalyst, shows evidence of only a small amount of ionic decomposition.

THE products and kinetics of the decomposition of benzoyl peroxide under various conditions have been frequently investigated (see Barnett and Vaughan, *J. Phys. Chem.*, 1947, **52**, 926, for references). It is now generally considered that, in most solvents, the first step is a unimolecular fission into free radicals, followed by a chain decomposition of the peroxide (Bartlett and Nozaki, *J. Amer. Chem. Soc.*, 1946, **68**, 1686; 1947, **69**, 2299). However, Bartlett and Leffler (*ibid.*, 1950, **72**, 67) found that some acid-catalysed ionic decomposition can probably occur, since addition of acetic, dichloroacetic, or trichloroacetic acid increases the rate of decomposition in benzene at 70° in that order, and addition of sulphuric acid increases the rate in acetic acid, though the effect is very much smaller than for *p*-anisoyl *p*-nitrobenzoyl peroxide (Leffler, *loc. cit.*).

Mixtures of organic solvents and water should provide favourable media for ionic decomposition. The only mention of the effect of water on the decomposition of benzoyl peroxide is by Gelissen and Hermans (*Ber.*, 1925, **58**, 765) : addition of water to *iso*butyl alcohol used as the solvent is stated to increase the yield of aldehyde and benzoic acid. It is difficult to find suitable compounds which are miscible with water. However, the effect of water on the decomposition of benzoyl peroxide in acetic acid, pyridine, phenol, and benzyl alcohol has been investigated, iodometric titration being used for rate measurements.

In phenol and phenol–water mixtures the overall decomposition was of the first order, the rate constant increasing slightly with increased initial concentration in the dry solvent at 68·5° ($k$ = 0·0322, 0·0351, and 0·0376 min.$^{-1}$ for 0·0165M-, 0·0331M-, and 0·0496M-solutions, respectively). Identical results, within the experimental error, were obtained on using fresh B.P. phenol directly or after further purification. Oxygen had no effect, but addition of water decreased the rate of decomposition, and $k$ was approximately proportional to the molar fraction of phenol in the solvent mixture, as shown in the following table. The

*Decomposition of benzoyl peroxide in phenol and phenol–water.*

*Temp.* = 67·6°. *Initial concn.* = 0·033M.

| | | | | | |
|---|---|---|---|---|---|
| (*a*) $k$ (min.$^{-1}$) | 0·0318 | 0·0274 | 0·0207 | 0·0138 | 0·00967 |
| (*b*) Phenol concn. (mole %) | 100 | 82·7 | 67·9 | 44·5 | 23·0 |
| $10^4(a)/(b)$ | 3·2 | 3·3 | 3·0 | 3·1 | 4·2 |

overall activation energies of decomposition in phenol and phenol + 55·5 mole-% water were practically the same, *viz.*, 19·6 and 19·8 kcal./g.-mol., respectively [Fig. 1(*b*) and (*c*)], which are much lower than the 30—32 kcal. found when relatively inert solvents are used (Barnett and Vaughan, *loc. cit.*). It seems, therefore, that the water was merely acting as

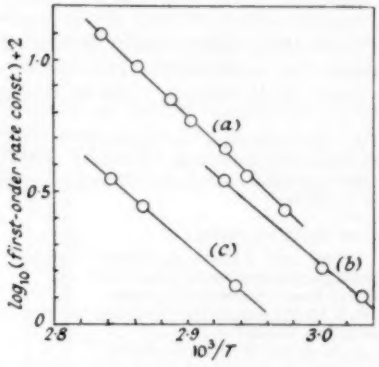



FIG. 1. *Arrhenius plots for decomposition of benzoyl peroxide in* (*a*) *pyridine* +74·7 *mole-%* $H_2O$, (*b*) *dry phenol*, (*c*) *phenol* +55·5 *mole-%* $H_2O$. *Initial concn.* = 3·31 × 10$^{-2}$M.

an inert diluent, and that the results are in accordance with Bartlett and Nozaki's suggestion (*loc. cit.*) that in phenol the high rate is due to a very rapid induced decomposition of the peroxide by $C_6H_5O\cdot$ radicals.

The decomposition in the other solvents, with or without added water, appears to be more complex, and the results are difficult to interpret. Under most conditions the reaction tended to a higher order than unity, although the rate again increased with increasing initial concentration (Figs. 2—5). The course of the decomposition in benzyl alcohol in an inert atmosphere was only slightly affected by the addition of up to 33 mole-% of water (Fig. 2). In the presence of oxygen the peroxide content of the solution reached a steady value, which was higher (25% compared with 52·4% apparent decomposition) in the alcohol–water mixture, and it seems likely that oxidation of the solvent was occurring, possibly to give $C_6H_5\cdot CH(OH)\cdot O\cdot OH$ (cf. Barnett and Vaughan, *loc. cit.*, pp. 945, 953). In acetic acid and pyridine, however, the rate of decomposition was considerably increased by the addition of water (Figs. 4 and 5), and so the reaction in these solvents was more extensively investigated.

Fig. 3 shows some sample results at 79·8° in dry acetic acid. The plot of $\log_{10}[Bz_2O_2]$ against time was not always reproducible at the same initial concentration in the presence of nitrogen, and depended on the specimen of acid used. Two portions from the same specimen, however, gave nearly identical curves. In freshly purified acid, the initial rate was about the same, but the curves "tailed off" at different stages in the decomposition. In the purest acid the decomposition remained approximately of first order for the longest time, and it was noticed that as the "tail off" occurred the solution began to become yellow. The difference in initial concentration being taken into account, the results agree

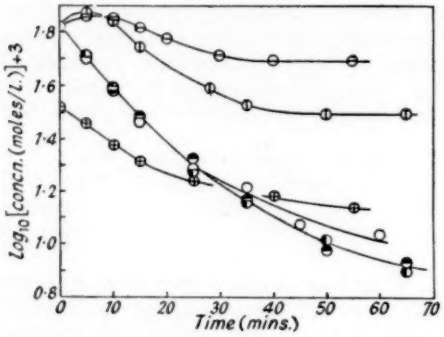



FIG. 2. *Effect of water and oxygen on the decomposition of benzoyl peroxide in benzyl alcohol. Temp.* = 79·2°. *Initial concns.* = $6·61 \times 10^{-2}$ *and* $3·31 \times 10^{-2}$M.

$N_2$ *passed through solution*

○, ⊕, *dry benzyl alcohol.*
◐, BzOH + 19·2 *mole-*% $H_2O$.
◓, BzOH +33·1 *mole-*% $H_2O$.

$O_2$ *passed through solution:*

◍, *as for* ○.
⊖, *as for* ◓.

quite well with that of Bartlett and Nozaki (*loc. cit.*). When analytical-grade glacial acetic acid from a Winchester bottle which had been opened some time previously was used directly as the solvent, the rate of reaction was greatly reduced. The decomposition became of first order and a yellow colour developed almost immediately. The presence of oxygen had exactly the same effect. The rate was reduced to the same value and was unaffected by the purity of the solvent. The decomposition was also retarded by the reaction products

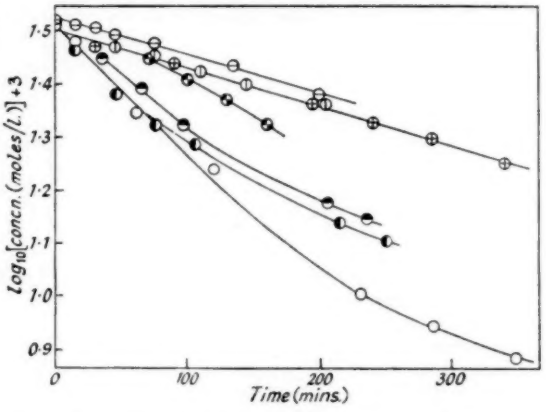
FIG. 3. *Decomposition of benzoyl peroxide in dry acetic acid. Temp.* = 79·8°. *Initial concn.* = $3·35 \pm 0·05 \times 10^{-2}$M.

$N_2$ *passed through solution:*

○, *Griffin and Tatlock analytical-grade glacial acetic acid, twice fractionated and fractionally frozen.*
◐, *B.D.H. "AnalaR" glacial acetic acid, fractionated.*
◓, *as for* ◐ *but fractionally frozen.*
⊖, *G. and T. analytical-grade acid from old, opened Winchester.*
◒, *as for* ○, *but containing yellow final solution.*

$O_2$ *passed through solution:*

⊕, *as for* ○.
◍, *as for* ⊖.




from the oxygen-retarded experiments. The yellow colour faded and the rate increased as the reaction proceeded. However, at 100° in the presence of nitrogen, results with different specimens of acid were identical, and even after 95% decomposition the yellow colour was very faint. Oxygen again reduced the rate, the reaction becoming of first order, but the presence of reaction products had only a slight effect.

It seems likely that the fall off from the initial first-order rate in the later stages of the decomposition was due to the formation of a yellow secondary oxidation product, which retarded the reaction. Oxygen or a trace of impurity in the acid caused the formation of

the yellow substance immediately. This substance may be a quinone, for these are known to inhibit the decomposition of benzoyl peroxide (Breitenbach and Kindl, *Monatsh.*, 1950, **81**, 1145). The retardation was probably due to partial or complete suppression of the

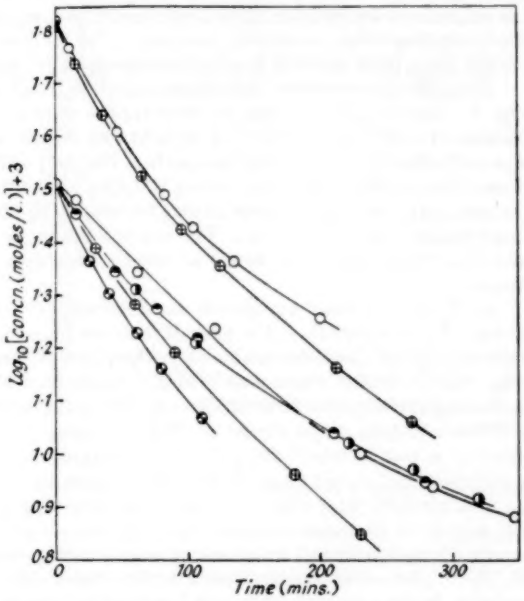



FIG. 4. *Effect of addition of water on the decomposition of benzoyl peroxide in acetic acid. Temp.* = 79·8°. *Initial concns.* = 6·71 × $10^{-2}$ *and* 3·30 × $10^{-2}$M. $N_2$ *passed through solution.*

○, *dry acid as for curve* ○, *Fig.* 3.

*Others : acetic acid–water.*

◑, 6·1 *mole-%.*
◓, 11·7 *mole-%.*
⊕, 44·2 *mole-%.*
◔, 67·9 *mole-%.*

chain reaction, since the time of half-change, ~340 mins. at 79·8° in the presence of oxygen, is roughly the same as $t_{\frac{1}{2}}$ for the fully inhibited decomposition in dioxan at 80° (Swain, Stockmayer, and Clarke, *J. Amer. Chem. Soc.*, 1950, **72**, 5426), and Bartlett and Nozaki (*loc. cit.*) found that, acetic anhydride, a solvent similar to acetic acid, being used, the

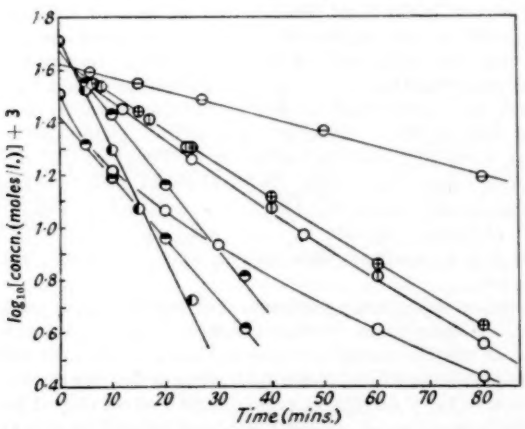



FIG. 5. *Decomposition of benzoyl peroxide in pyridine and pyridine–water. Temp.* = 79·7°. *Initial concns.* = 5·29 × $10^{-2}$ *and* 3·31 × $10^{-2}$M.

$N_2$ *passed through solution :*

○, *pure pyridine.*
①, *pyridine containing products of decomposition.*
⊕, *pyridine* +15·6 *mole-%* $H_2O$.
◓, *pyridine* +53·0 *mole-%* $H_2O$.
◐, *pyridine* +74·7 *mole-%* $H_2O$.

$O_2$ *passed through solution :*

⊖, *pure pyridine.*

decomposition was retarded by the reaction products, the chain reaction being reduced to approximately one-third of the original. Also, the overall activation energy of decomposition in the presence of oxygen, 31·8 kcal./mole ($k$ = 0·00166 min.$^{-1}$ at 79·75° and 0·0115 min.$^{-1}$ at 100·1°), agrees quite well with the value found in the presence of a stable

free radical in a number of solvents (Bawn and Mellish, *Trans. Faraday Soc.*, 1951, **47**, 1216). At 100° the stability of the inhibitor seems to have been less than at 80°.

Since, when the acetic acid contained 67·9 mole-% of water, neither the purity of the acid nor the presence of oxygen had any marked effect on the rate of decomposition, and the solution never became more than faintly yellow, the water must largely have prevented the formation of the retarding product. The increase in the rate on the addition of water cannot have been entirely due to this because the initial rates were also increased.

The influence of water and oxygen on the rate of decomposition in pyridine is shown in Fig. 5. In dry pyridine and an atmosphere of nitrogen, after a short period the reaction became of nearly first order. A dark brown colour developed in the solution, but the rate was not affected by reaction products. Bartlett and Nozaki (*loc. cit.*) found a somewhat slower rate of decomposition, owing possibly to a difference in purity of the pyridine used. Oxygen retarded the decomposition, causing it to become of first order, probably for the same reason as in acetic acid. The overall activation energy, 31·9 kcal./mole ($k = 0{\cdot}0123$ min.$^{-1}$ at 79·65° and 0·155 min.$^{-1}$ at 100·4°), was again approximately that for unimolecular fission.

Addition of a small amount of water decreased the rate slightly, but a greater quantity increased it considerably, the overall reaction becoming of first order. The overall activation energy of decomposition in pyridine + 74·7 mole-% of water was 22·0 kcal./mole [Fig. 1(*a*)]. Under these conditions $k$ decreased slightly, rather than increased, with increasing initial concentration ($k = 0{\cdot}125$, 0·112, and 0·112 min.$^{-1}$ for 0·0331, 0·0527, and 0·0870M-solutions respectively at 79·7°). Thus, although oxygen had a small retarding effect ($k = 0{\cdot}0927$ min.$^{-1}$ for a 0·0527M-solution), the value of 22·0 kcal. probably refers to a side reaction, rather than to any rapid chain decomposition.

It is unlikely that the increase in the rate of decomposition on the addition of water was due to an increased induced decomposition by solvent radicals, since the dissociation energy of the first O–H bond in water is very high, ~118 kcal. (Szwarz, *Chem. Reviews*, 1950, **47**, 75). Two other possibilities are that some ionic decomposition or hydrolysis of the peroxide in the presence of the acid or base was occurring :

$$[Ph{\cdot}CO{\cdot}O{\cdot}]_2 + H_2O = Ph{\cdot}CO{\cdot}O{\cdot}OH + Ph{\cdot}CO_2H \text{ *}$$

If perbenzoic acid was formed it would have decomposed very rapidly at the temperatures used, probably giving a high proportion of benzoic acid, since at 0° Meyer (*J. Biol. Chem.*, 1933, **103**, 25) found that it was unstable in the presence of water and acids or pyridine. A study of the variation on addition of water, in the final yield of carbon dioxide and non-volatile acid, produced in the decomposition, and in the efficiency of the peroxide as a polymerisation catalyst, was made in the hope of finding whether the increase in rate was due to either possibility. It would be expected that any ionic decomposition would have led to a decrease in the polymer formed, whereas hydrolysis would probably have led to an increased yield of acid and a decreased yield of carbon dioxide. The following table shows the results. These do not conflict with those obtained by Barnett and Vaughan (*loc. cit.*) by using acetic acid, and by Overhoff and Tilman (*Rec. Trav. chim.*, 1929, **48**, 993) by using pyridine as solvent, since increase of temperature is known to lead to an increased yield of carbon dioxide. The very high yield of carbon dioxide in acetic acid, despite the high yield of non-volatile acid, suggests that acetoxy-radicals were formed from the solvent and decarboxylated to some extent on reaction.

Some hydrolysis was probably occurring in aqueous pyridine, and possibly the activation energy of 22·0 kcal. refers to this. If some ionic decomposition and hydrolysis were occurring in aqueous acetic acid, both of which caused a decrease in the carbon dioxide yield but influenced the acid yield in opposite direction, this might account for the results. On the other hand, the peroxide appeared to be, if anything, more efficient as a catalyst for the polymerisation of acrylonitrile in the aqueous acid. However, it can be said that, even

* A referee has pointed out that other equilibria, *e.g.*,

$$[Ph{\cdot}CO{\cdot}O{\cdot}]_2 + AcOH \rightleftharpoons Ph{\cdot}CO{\cdot}O{\cdot}OAc + Ph{\cdot}CO_2H$$

might have occurred, giving other per-compounds, and might possibly explain the more anomalous decomposition rates.

under very favourable conditions, the amount of heterolytic fission of benzoyl peroxide was small. The increase in the rate of decomposition in aqueous acetic acid and pyridine was probably due to a combination of three factors—the partial suppression of the formation

| Temp. | Solvent | Initial concn. of $Bz_2O_2$, M | Yield of products: Moles $CO_2$/ mole $Bz_2O_2$ | Equivs. acid/ mole $Bz_2O_2$ | G. of polymer from solvent + 8 vol.-% acrylonitrile |
|---|---|---|---|---|---|
| 100·0° | AcOH | 0·0165 | — | — | 0·516 |
| | | 0·0331 | 1·42 | 1·51 | 0·731 |
| | | 0·0496 | 1·34 | 1·63 | — |
| | AcOH + 67·9 mole-% $H_2O$ | 0·0165 | — | — | 0·659 |
| | | 0·0331 | 1·19 | 1·55 | 0·783 |
| | | 0·0496 | 1·12 | 1·50 | — |
| 79·7 | $C_5H_5N$ | 0·0165 | — | — | 0·117 |
| | | 0·0331 | 0·51 | 1·18 | 0·526 |
| | | 0·0496 | 0·54 | 1·19 | — |
| | $C_5H_5N$ + 74·7 mole-% $H_2O$ | 0·0165 | — | — | 0·160 |
| | | 0·0331 | 0·39 | 1·32 | 0·326 |
| | | 0·0496 | 0·46 | 1·41 | — |

of a retarding secondary product, together with some hydrolysis and a little ionic decomposition. More elaborate methods are probably needed, however, to disentangle the complex side-reactions which seem to occur during the decomposition of the peroxide in many solvents.

## Experimental

An all-glass apparatus was used. A very slow stream of nitrogen or oxygen was passed through 25 ml. of peroxide solution, heated at constant temperatures (±0·05°) by the vapours of refluxing liquids. Samples were withdrawn at suitable intervals and analysed by Skellon and Wills's iodometric method (*Analyst*, 1948, **73**, 78). Blank titres were less than 0·1 ml.

B.D.H. Benzoyl peroxide was precipitated twice from hot chloroform solution by methanol. The dry product (m. p. 103·1—103·3°) released 98—99·5% of the theoretical amount of iodine from acidified potassium iodide. B.P. Phenol from freshly opened bottles was used directly or fractionally melted. Pyridine and benzyl alcohol were dried and fractionally distilled twice. "Analar" or analytical-grade acetic acid was used directly or fractionally distilled with 1% of acetic anhydride, and then sometimes fractionally frozen. The solvents contained <0·01—0·1% water determined by the Karl Fischer method as modified by Smith, Bryant, and Mitchell (*J. Amer. Chem. Soc.*, 1939, **61**, 2407). B.D.H. Acrylonitrile was distilled under a high vacuum and then redistilled before each experiment. Nitrogen from a cylinder was passed over red-hot copper and through a liquid-oxygen trap to remove oxygen, carbon dioxide, and water vapour. Oxygen from a cylinder was passed through a liquid-oxygen trap.

To estimate carbon dioxide and non-volatile acids formed in the decomposition, samples were not removed, and the issuing gases were passed through a calcium chloride tube, a phosphoric oxide tube and a soda-lime tube, until the weight of the last became constant. Blank experiments showed a very small increase in weight (<1 mg.) and this was allowed for. The final solutions were allowed to evaporate slowly, and the solids left were dried *in vacuo* over phosphoric oxide for 2 hours. Each dry product was taken up in 10 ml. of aclohol and titrated with 0·1N-sodium hydroxide with phenolphthalein as indicator. Parallel experiments with known amounts of benzoic acid showed that 94—95% was recovered in this way.

Solutions of benzoyl peroxide in 2 ml. of acrylonitrile and 23 ml. of solvent, in the presence of nitrogen, began to form fluffy insoluble polymer almost immediately. When decomposition of the peroxide was known to be complete, the mixture was transferred to a beaker and 25—50 ml. of water were added. The polymer was filtered off through a sintered-glass crucible, washed thoroughly with water, and dried to constant weight at 100°.

The author thanks Professor C. E. H. Bawn (Liverpool) and Dr. J. Thomas for helpful comments, and W. B. Fordyce and G. D. Aitchison for assistance with part of the experimental work.

King's Buildings, Edinburgh, 9. [*Received, February 26th*, 1952.]

## 569. *The Action of Formaldehyde on Proteins. Part II.* Some Reactions of N-Hydroxymethylamides.*

By R. D. Haworth, D. H. Peacock, W. R. Smith, and (in part) R. MacGillivray.

The equilibria between formaldehyde, amides, and *N*-hydroxymethylamides have been studied under alkaline conditions. In acid solution the methylenebisamides are formed (Einhorn, *Annalen*, 1905, **343**, 207; 1908, **361**, 113). It has been found that *N*-hydroxymethylamides condense with primary alcohols to form *O*-ethers, with thiols to form sulphides, and with carboxylic acids to form esters; with primary amines two molecules of the *N*-hydroxymethyl compound combine to form tertiary amino-derivatives. The reaction with secondary amines (Einhorn, *loc. cit.*) has been confirmed. 1 : 4-Bishydroxymethyl-2 : 5-diketopiperazine is not formed in acid solution, and under acid conditions does not condense with amides but loses formaldehyde which forms a methylenebisamide. *N*-Hydroxymethylamides do not condense with peptides. These results are applied to a study of the nature of the products formed by the action of formaldehyde upon proteins.

Haworth, MacGillivray, and Peacock (Part I*) showed that several proteins when treated with formaldehyde in alkaline solution gave products which condensed with β-naphthol and with 6-bromo- and 3 : 6-dibromo-2-naphthol to form compounds yielding 1-aminomethyl-2-naphthol or its derivatives on hydrolysis. Ground-nut protein insolubilised with formaldehyde under acid conditions (Traill, *Chem. and Ind.*, 1945, 58; 1950, 23) condensed with a much smaller amount of 6-bromo-2-naphthol and, if the acid-treated protein had been previously boiled with water, the amount taken up was negligible. Evidence was adduced that amongst the first products of the action of formaldehyde on alkaline solutions were *N*-hydroxymethylamides or labile derivatives, and it seemed feasible that more complicated reactions took place during treatment with formaldehyde under acid conditions, as a result of the condensation of the *N*-hydroxymethylamides with other groupings present in the protein molecules. The object of the present work was to examine the reactions of *N*-hydroxymethylamides with model substances containing groupings such as hydroxyl, thiol, amino-, carboxyl, amide, or peptide, which are present in protein molecules, and to compare the stability of the products with those of acid–formaldehyde-treated proteins. The *N*-hydroxymethylamides (I) were prepared by the action of formaldehyde on a wide variety of amides in the presence of aqueous potassium carbonate, and included those from benzamide (I; R = Ph), phenylacetamide, β-phenyl-

(I) $R\cdot CO\cdot NH\cdot CH_2OH$ $R\cdot CO\cdot NH\cdot CH_2\cdot NH\cdot CO\cdot R$ (II)

propionamide, hippuramide, phenylacetylglycine amide, *N*-phenylacetyl-DL-alanine amide, and succinamide. All these *N*-hydroxymethylamides condensed with β-naphthol in alcoholic hydrochloric acid, and the 1-acylaminomethyl-2-naphthol derivative gave 1-aminomethyl-2-naphthol on hydrolysis with hydrochloric acid. The *N*-hydroxymethylamides are stable in neutral aqueous solution at room temperature but the reaction between the original amides and formaldehyde in presence of potassium carbonate is reversible. The formation and dissociation of a number of substances of type (I) have been examined by determination of the liberated formaldehyde with 2 : 4-dinitrophenylhydrazine (Lamberton, Lindley, Owston, and Speakman, *J.*, 1949, 1641), with the results given in Table 1. When formaldehyde in aqueous potassium carbonate was mixed with benzamide, equilibrium was reached in 48 hours and the reciprocal of the equilibrium constant was $1{\cdot}6 \times 10^{-5}$; for *N*-phenylacetylglycine amide, the time was 70 hours and the above reciprocal was $4{\cdot}5 \times 10^{-5}$. These figures were of the same order as the dissociation constants, and better agreement could not be expected. The hydroxymethyl derivatives of acylated amino-amides such as phenylacetylglycine amide are somwhat less stable than those of benzamide and similar amides, and the stability of the bishydroxymethyl derivatives of succinamide

* *J.*, 1950, 1493, is considered to be Part I of this series.

TABLE 1. *Dissociation of* N-*hydroxymethylamides.*

0·5 G. of hydroxymethyl derivative and 0·02 g. of potassium carbonate in 50 c.c. of water at room temperature.

| R in (I) | Percentage of total formaldehyde liberated after: 5 hr. | 17 hr. | 51 hr. | 120 hr. | 212 hr. | 15 days | $10^5K$ |
|---|---|---|---|---|---|---|---|
| Ph | 23·6 | 38·1 | 43·3 | 44·5 | — | 44·8 | 2·8 |
| ,, ($K_2CO_3$, 0·04 g.) | 33·5 | 39·9 | 40·4 | 42·3 | — | 42·2 | — |
| $CH_2Ph$ | — | 37·7 | — | 42·5 | 42·2 | — | 2·3 |
| | 54 hr. | 98 hr. | 142 hr. | 21 days | | | |
| $CH_2Ph{\cdot}CO{\cdot}NH{\cdot}CH_2$ | 49·5 | 53·3 | 53·8 | 53·2 | — | — | 7·3 |
| $CH_2Ph{\cdot}CO{\cdot}NMe{\cdot}CH_2$ | 26 hr. | 68 hr. | 115 hr. | 21 days | | | |
| Succinamide | 6·8 | 9·4 | 9·9 | 10·0 | | | |

is noteworthy. In acid solution the *N*-hydroxymethylamides are converted into methylenebisamides of type (II), and these compounds are also obtained by condensing formaldehyde and amides at pH 0. In Table 2 the figures relating to the decomposition of *N*-hydroxymethylamides in acid solution are given.

TABLE 2. *Decomposition of* N-*hydroxymethylamides in acid.*

0·5 G. of hydroxymethyl derivative in 50 c.c. of 2N-hydrochloric acid.

| R in (I) | Percentage of formaldehyde split off: 20 hr. | 50 hr. | 112 hr. | 250 hr. | 16 days | 21 days |
|---|---|---|---|---|---|---|
| Ph | 18·3 | 35·4 | 41·6 | 43·1 | — | — |
| $CH_2Ph{\cdot}CO{\cdot}NH{\cdot}CH_2$ | 8·8 | — | 30·4 | 40·7 | — | — |
| Succinamide | 10·1 | 19·1 | 24·5 | — | — | 37·8 |
| Acetate of (I; R = $CH_2Ph$) | 13·8 | 24·3 | 44·8 | 65·0 | 73·0 | — |

Methylenebisamides (II) are stable compounds; they are not attacked by N-sodium hydroxide during 1 hour at 90° and are relatively stable towards acids, but in 10 hours' boiling with 4% alcoholic hydrochloric acid and β-naphthol the formaldehyde is removed as methylenebis-β-naphthol.

During some of the experiments outlined above it was found that *N*-hydroxymethylamides frequently yielded *O*-ethers when treated with alcohols in the presence of acids and in some cases the reactions occurred with considerable ease. Similar ready *O*-alkylation is familiar with carbinol bases (Robinson and McLeod, *J.*, 1921, 1470) and the alkylation of compounds of type (I) has previously been reported for a few cases (Kadowski, *Bull. Chem. Soc. Japan*, 1936, **11**, 248; Sorenson, U.S.P. 2,201,927; Allbrecht, Frei, and Sallmann, *Helv. Chim. Acta*, 1941, 233E). The *O*-ethers have now been prepared from a variety of *N*-hydroxymethylamides and primary alcohols, including *N*-benzoylserine, but reaction has not been observed with secondary or tertiary alcohols. The *O*-ethers showed reactivity resembling that of the parent *N*-hydroxymethylamide. They were more slowly decomposed to the parent alcohol, amide, and formaldehyde by boiling water or by N-sodium hydroxide at room temperature, and they were converted into methylenebisamides by warming or by long contact in the cold with aqueous hydrochloric acid. They underwent alcoholysis when warmed with another alcohol in the presence of concentrated hydrochloric acid, and with β-naphthol they condensed as readily as did the *N*-hydroxymethylamide and gave the same products. A few sulphides have also been prepared by condensation with thiols; they were decidedly more stable, and reacted with β-naphthol only after 3 hours' boiling, compared with 20 minutes for the corresponding *O*-ethers.

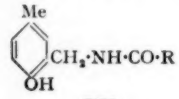
Compounds of type (III) were prepared by Einhorn (*Annalen*, 1905, **343**, 207; 1908, **361**, 113) from amides, formaldehyde and secondary amines, or from *N*-hydroxymethylamides and secondary amines. The reaction between methylenebis-*sec.*-amines and amides can also be employed, but we find the best yields are obtained by the first of the three methods. The reactions with primary amines and *N*-hydroxymethylamides were less well-known. Fraenkel-Conrat and Olcott (*J. Amer. Chem. Soc.*, 1948, **70**, 2673) described the production of an unstable compound of type (IV) by condensing formaldehyde with acetamide and alanine, and similar structures were proposed by Sallemann and Graenacher (U.S.P. 2,448,125) for the basic products obtained by condensing *N*-hydroxy-

methylamides with aliphatic amines and formaldehyde in acetic acid solution. The reaction between *N*-hydroxymethylamides and primary amines has now been investigated more thoroughly. For *N*-hydroxymethyl derivatives of benzamide, phenylacetamide, and β-phenylpropionamide and methylamine, condensation was effected in water at

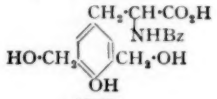
$R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}NR'R''$ (III) $\quad R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}NH{\cdot}R'$ (IV) $\quad (R{\cdot}CO{\cdot}NH{\cdot}CH_2)_2NR'$ (V)

100° for 12 hours or preferably in glacial acetic acid at 75° for 45 minutes. The analytical data and molecular weights of the crystalline products proved that *N*-hydroxymethylamides reacted with primary amines to give products of type (V), and not of type (IV) as previously suggested. Burke's observation (*ibid.*, 1947, **69**, 2136) that symmetrical bishydroxymethylurea reacted with a primary amine to form a cyclic triazine derivative is, however, quite consistent with the production of compounds of type (V). The compounds of types (III) and (V) are fairly stable in alkaline solution at room temperature, and they do not condense with β-naphthol under the conditions which are effective with *N*-hydroxymethylamides. The secondary amine condensation products of type (III) are decomposed more slowly than primary amine products of type (V) by boiling water or aqueous acid.

$R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}OAc$ (VI)



(VII)

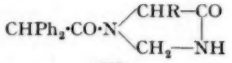


(VIII)

During the examination of the reactions of primary amines with *N*-hydroxymethylamides in acetic acid, esters of type (VI) were encountered. They were, however, more conveniently prepared by heating *N*-hydroxymethylamides with glacial acetic acid at 75°. They are not rapidly decomposed by boiling water, and towards acids and alkalis they are rather more stable than the *N*-hydroxymethylamides, but they readily react with β-naphthol under the same conditions, and to give the same products, as the corresponding compounds of type (I).

*N*-Hydroxymethylamides readily condense with active phenols and naphthols (Einhorn, *loc. cit.*) and it has now been shown that *N*-hydroxymethyl-benzamide and -phenylacetamide react normally with *p*-cresol in alcoholic hydrochloric acid, to give derivatives of type (VII). *N*-Benzoyltyrosine therefore represented an interesting model, but all attempts to effect its reaction with *N*-hydroxymethyl-benzamide or -phenylacetamide failed. Under the acidic conditions employed the first gave methylenebisbenzamide and the second gave a mixture of methylenebisphenylacetamide and *N*-ethoxymethylphenylacetamide; *N*-benzoyltyrosine was recovered in both cases. *N*-Benzoyltyrosine did not react with formaldehyde in aqueous or alcoholic hydrochloric acid, but in sodium hydroxide solution the 3 : 5-dihydroxymethyl derivative (VIII) was produced, similar to the compound obtained under these conditions from *p*-cresol (Ullmann and Brittner, *Ber.*, 1909, **42**, 2539).

$R{\cdot}CO{\cdot}NH{\cdot}R'$ (IX) $\quad R{\cdot}CO{\cdot}NH{\cdot}CHR'{\cdot}CO{\cdot}NR''R'''$ (X)



(XI)

This failure to bring about reaction between formaldehyde or *N*-hydroxymethylamides with the amide-nitrogen atom of *N*-benzoyltyrosine illustrates the general lack of reactivity which we have observed with monoacylated primary amines of type (IX). Although diketopiperazine (Cherbuliez and Feer, *Helv. Chim. Acta*, 1922, **5**, 678) and polyamides of the Nylon type (Lewis and McCreath, B.P. 582,517; Cairns, Foster, Larcher, Schneider, and Schreiber, *J. Amer. Chem. Soc.*, 1949, **71**, 651) have been shown to give the expected *N*-hydroxymethyl derivatives, Fraenkel-Conrat, Coober, and Olcott (*J. Amer. Chem. Soc.*, 1945, **67**, 950) found that the silk fibroin and polyglutamic acid did not condense with formaldehyde at pH 4. A number of peptides of type (X; *e.g.*, R = Ph or $CH_2Ph$, R′ = H or Me, and NR″R‴ = morpholino, NHEt, or $NEt_2$) have been synthesised as described

in the Experimental section, but these substances did not react with formaldehyde under a wide variety of conditions, including the use of potassium carbonate or pyridine as basic, and hydrochloric or formic acid as acid, catalyst, with water, alcohol, or dioxan as solvent. As it was thought possible that the products might be decomposed during attempted isolation, the reaction mixtures from formaldehyde and amides of types (IX) and (X) were treated with β-naphthol in acid solution, but no derivative of a substituted 1-aminomethyl-2-naphthol could be isolated as such or as the toluene-*p*-sulphonyl derivative. In addition, analytical determination with 2 : 4-dinitrophenylhydrazine showed no formaldehyde uptake after formaldehyde and amides of types (IX) and (X) had been in contact for 21 days at room temperature. All the amides of types (IX) and (X) which we have examined fail to react with *N*-hydroxymethylamides, and in this connection it is particularly interesting that the *N*-hydroxymethyl derivatives of phenylacetylglycine amide and phenylacetyl-DL-alanine amide cannot be converted into cyclic derivatives of type (XI) by intramolecular condensation of the *N*-hydroxymethylamide group with the peptide imide group. In 1 : 4-bishydroxymethyl-2 : 5-diketopiperazine the hydroxymethyl group is already linked to a peptide group. When this was treated with phenylacetamide in acid solution the formaldehyde was split from the diketopiperazine and formed the methylenebisamide of phenylacetic acid.

For the reactions occurring during formaldehyde treatment of proteins condensation with amino-groups has frequently been postulated. Secondary amines and formaldehyde usually give methylenebisamines, but the reaction with primary amines has been variously interpreted, and methylenebisamine (Kempf, *Annalen*, 1890, **256**, 219), hydroxymethylamine, and methyleneimine structures (Henry, *Bull. Acad. Roy. Belg.*, 1894, **28**, 355) have been suggested. Our experiments with benzylamine and formaldehyde indicate that the product, m. p. 50°, is a cyclic trimer of *N*-methylenebenzylamine, and the salts, described by Kempf (*loc. cit.*) are in fact benzylamine salts. The cyclic trimers and methylenebisamines are stable to cold alkalis and are rapidly decomposed by dilute mineral acids, and it is unlikely that these compounds play any part in the acid-insolubilising reactions. On the other hand the experimental results described in Part I and in this and the following paper provide convincing evidence of the importance of reactions at amide centres of the protein molecule. Other centres may also be involved and, as a result of these experiments with model substances, deductions may be now be made concerning the reactions occurring during the insolubilising of proteins with formaldehyde in acid solution.

Macromolecules are usually more sluggish in their reactions than less complex polymers, and as it is not always possible to draw firm conclusions about the behaviour of macromolecules from experiments with simple compounds, the following conclusions must be regarded as tentative: (1) *N*-Hydroxymethylamides are not likely to persist to any appreciable extent in acid-treated proteins; and small amounts present would contribute to the reaction with β-naphthol and would be eliminated by boiling water. (2) There is no evidence for the formation of methylene bridges between amide and peptide groups, or between amide and aromatic nuclei, *e.g.*, tyrosine. (3) Methylene bridges between the amide groups and hydroxyl, thiol, carboxyl, or primary or secondary amino-groups may be present in acid-treated unboiled fibre and these linkages may be responsible for some of the formaldehyde eliminated during boiling with water, and for some of the reactions with β-naphthol. (4) Methylenebisamide linkages would probably be formed in the acid-insolubilising bath; the products would not liberate formaldehyde when boiled with water, would be relatively stable towards acids and alkalis, and would not yield 1-aminomethyl-2-naphthol derivatives.

## Experimental

*Equilibria between Amides, Formaldehyde, and Water.*—To a solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid (Brady, *J.*, 1931, 757) was added sufficient formaldehyde solution to give a faint precipitate. The reagent was then kept at the ordinary temperature for 48 hours and filtered. It was found that 0·2% formaldehyde solution precipitated 99% of the formaldehyde as the dinitrophenylhydrazone after 15 minutes; Lamberton *et al.* (*loc. cit.*) used a reaction time of 5 hours, during which the equilibria examined in this paper would have

appreciably changed. 0·2% Formaldehyde solution showed no loss of strength after 7 days in 2N-hydrochloric acid or 0·08% potassium carbonate solution. In N-sodium hydroxide a fall in formaldehyde content was observed after 48 hours. The solubility of the dinitrophenylhydrazone in 2N-hydrochloric acid at ordinary temperature was 0·5 mg./10 c.c.

*Dissociation of N-Hydroxymethylamides in Alkaline Solution.*—In a typical experiment the following results were obtained :

*N*-Hydroxymethylbenzamide (0·5429 g.), potassium carbonate (0·02 g.) made up to 50 c.c. in water; 10 c.c. samples were withdrawn.

| Time after mixing (hrs.) | 5 | 17 | 51 | 120 | 15 days |
|---|---|---|---|---|---|
| Wt. of dinitrophenylhydrazone (g.) | 0·0387 | 0·0624 | 0·0709 | 0·0731 | 0·0734 |
| Total formaldehyde set free (%) | 23·6 | 38·1 | 43·3 | 44·5 | 44·8 |

Whence $K = 2{\cdot}8 \times 10^{-5}$.

*Combination of Formaldehyde with Amides in Alkaline Solution.*—A standardised solution of formaldehyde (2·55 g./100 c.c.) was used and the equilibrium found in the usual way :

Benzamide. Time to reach equilm. ..... 48 hrs., whence $1/K = 1{\cdot}6 \times 10^{-5}$.
Phenylacetylglycine amide. Time to reach equilm. ..... 70 hrs., whence $1/K = 4{\cdot}5 \times 10^{-5}$.

*Decomposition of N-Hydroxymethylamides in Acid Solution.*—The methylenebisamide formed was removed by filtration before the liberated formaldehyde was determined. The results are given in Table 2. When the *N*-hydroxymethylbenzamide solution was heated for 15 minutes in a water-bath with 2 : 4-dinitrophenylhydrazine, 91·2% of the total formaldehyde present was precipitated as the hydrazone; *N*-hydroxymethylphenylacetylglycine amide, treated similarly, gave 91·8% of formaldehyde.

*N-Hydroxymethylamides and their Reaction with β-Naphthol.*—*Phenylacetyl-DL-alanine ethyl ester* was prepared in a manner similar to the acid (see below), by using magnesium oxide to neutralise the hydrochloric acid liberated. It crystallised from ethanol or *cyclo*hexane in needles, m. p. 162° (Found : C, 66·3; H, 7·3; N, 6·2. $C_{13}H_{17}O_3N$ requires C, 66·4; H, 7·2; N, 6·0%). The *amide* was prepared from the ester (20 g.) in ammonia (*d* 0·880; 150 c.c.), stirred for 3 hours, further saturated with ammonia, and stirred for 2 hours. The product (12·6 g.) crystallised from water in prisms, m. p. 154—155° (Found : C, 64·4; H, 6·9; N, 13·4. $C_{11}H_{14}O_2N_2$ requires C, 64·1; H, 6·8; N, 13·3%).

*N-Hydroxymethylphenylacetylglycine amide.* Phenylacetylglycine amide (Hotter, *J. pr. Chem.*, 1888, **38**, 97) (10·0 g.), anhydrous potassium carbonate (0·4 g.), water (10 c.c.), and 40% aqueous formaldehyde were warmed in a water-bath until all was in solution. The *hydroxymethyl* derivative (10·0 g.) crystallised on cooling, and, recrystallised from water, had m. p. 144—148° (Found : C, 59·4; H, 6·2; N, 12·6. $C_{11}H_{14}O_3N_2$ requires C, 59·5; H, 6·3; N, 12·6%).

*N-Hydroxymethylphenylacetyl-DL-alanine amide*, prepared similarly in 87% yield, crystallised from water in plates, m. p. 137—140° (Found : C, 61·2; H, 6·7; N, 11·7. $C_{12}H_{16}O_3N_2$ requires C, 61·0; H, 6·8; N, 11·9%). *N*-Hydroxymethylhippuramide was prepared similarly but decomposed on attempted recrystallisation. *N-Hydroxymethyl-β-phenylpropionamide* crystallised from benzene or ethyl acetate in prisms, m. p. 98° (Found : C, 67·4; H, 7·0. $C_{10}H_{13}O_2N$ requires C, 67·1; H, 7·3%).

Condensation of the *N*-hydroxymethylamides with β-naphthol in alcoholic acid was effected as described by Einhorn (*Annalen*, 1905, **343**, 207); on hydrolysis in alcohol with concentrated hydrochloric acid all of the products described below gave the hydrochloride of 1-aminomethyl-2-naphthol. The following crystallised from ethanol: *1-N-Phenylacetylglycylamidomethyl-2-naphthol*, needles, m. p. 189° (Found : C, 72·2; H, 5·7; N, 7·9. $C_{21}H_{20}O_3N_2$ requires C, 72·4; H, 5·8; N, 8·1%). *1-Phenylacetyl-DL-alanylamidomethyl-2-naphthol*, needles, m. p. 200° (Found : C, 72·6; H, 5·9; N, 7·9. $C_{22}H_{22}O_3N_2$ requires C, 72·9; H, 6·1; N, 7·7%). *1-Hippuroylamidomethyl-2-naphthol*, prisms, m. p. 185—186° (Found : C, 71·6; H, 5·6; N, 8·1. $C_{20}H_{18}O_3N_2$ requires C, 71·9; H, 5·4; N, 8·4%). *1-β-Phenylpropionamidomethyl-2-naphthol*, prisms, m. p. 121—122° (Found : C, 78·3; H, 6·2; N, 5·1. $C_{20}H_{19}O_2N$ requires C, 78·6; H, 6·2; N, 4·6%).

*Methylenebisamides.*—These were prepared by dissolving the corresponding amides in a mixture of saturated sodium sulphate solution (860 c.c.), concentrated sulphuric acid (258 c.c.), and 40% formaldehyde solution (56 c.c.). The amide (0·5 g.) was mixed with the acid formaldehyde (5 c.c.) and warmed until solution was complete. The methylenebisamide separated on cooling. The compounds were also obtained when the *N*-hydroxymethylamides were warmed with 0·5N-hydrochloric acid until solution occurred, and

separated on cooling. The following crystallised [from acetic acid: *Methylenebis-phenylacetylglycylamide*, plates, m. p. 260° (Found: C, 63·5; H, 6·1; N, 14·1. $C_{21}H_{24}O_4N_4$ requires C, 63·6; H, 6·1; N, 14·2%). *Methylenebisphenylacetyl-*DL*-alanine amide*, prisms, m. p. 280° (decomp.) (Found: C, 64·9; H, 6·9; N, 13·3. $C_{23}H_{28}O_4N_4$ requires C, 65·1; H, 6·6; N, 13·2%). *Methylenebishippuramide*, plates, m. p. 258° (Found: C, 61·9; H, 5·8; N, 15·7. $C_{19}H_{20}O_4N_4$ requires C, 62·0; H, 5·5; N, 15·2%).

O-*Ethers* $R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}OR'$.—The ethers were prepared by warming a mixture of *N*-hydroxymethylamide (0·5 g.) with the appropriate alcohol (5 c.c.) and concentrated hydrochloric acid (0·1 c.c.) until solution occurred. The product usually separated on cooling, in some cases in a few minutes, in others only after several hours; in a few cases it was necessary to concentrate the solutions under reduced pressure. The ethers were also prepared by setting the mixed reactants aside for 18—20 hours at ordinary temperature. The following were prepared: N-*Methoxymethylphenylacetylglyine amide*, plates (from water), m. p. 146° (Found: C, 60·9; H, 6·7; N, 11·8. $C_{12}H_{16}O_3N_2$ requires C, 61·0; H, 6·8; N, 11·9%). N-*Ethoxymethyl-phenylacetylglycine amide*, plates (from water), m. p. 159° (Found: C, 62·2; H, 7·2; N, 11·7. $C_{13}H_{18}O_3N_2$ requires C, 62·4; H, 7·2; N, 11·2%), soluble in ethanol, hot acetone, benzene, chloroform, hot ethyl acetate, or pyridine, fairly soluble in dioxan, sparingly soluble in hot, light petroleum, insoluble in carbon tetrachloride and ether. [This ether (0·2 g.), when warmed with 0·5N-hydrochloric acid (2 c.c.), deposited the methylenebisamide on cooling. In N-sodium hydroxide at room temperature the ether set free the following percentages of total formaldehyde: 48 hours, 14·9; 9 days, 55·8.] N-n-*Propoxy-*, plates (from water), m. p. 145° (Found: C, 63·4; H, 7·3; N, 10·8. $C_{14}H_{20}O_3N_2$ requires C, 63·6; H, 7·6; N, 10·6%), N-iso-*butoxy-*, plates (from water), m. p. 120—121° (Found: C, 64·9; H, 7·9; N, 9·9. $C_{15}H_{22}O_3N_2$ requires C, 64·8; H, 7·9; N, 10·1%), and N-*benzyloxy-methylphenylacetylglycine amide*, leaflets (from aqueous ethanol), m. p. 138° (Found: C, 68·9; H, 6·2; N, 8·6. $C_{18}H_{20}O_3N_2$ requires C, 69·2; H, 6·4; N, 9·0%). [The benzyl ether (0·2 g.), ethyl alcohol (2 c.c.), and concentrated hydrochloric acid (0·1 c.c.) were warmed until all was in solution. The solid which separated on cooling melted at 157°, raised to 158° by crystallisation from water and unchanged after admixture with an authentic specimen of the ethyl ether.] N-*Ethoxy-*, prisms (from water), m. p. 128° (Found: C, 63·7; H, 7·5; N, 10·6. $C_{14}H_{12}O_3N_2$ requires C, 63·6; H, 7·6; N, 10·6%), and N-*benzyloxy-methylphenylacetyl-*DL*-alanine amide*, leaflets (from alcohol), m. p. 101° (Found: C, 69·8; H, 6·8; N, 8·3. $C_{19}H_{22}O_3N_2$ requires C, 69·9; H, 6·8; N, 8·6%) (this compound was obtained by neutralising the reaction mixture with potassium carbonate and removing the excess of benzyl alcohol by distillation under reduced pressure). N-*Methoxy-*, plates (from water), m. p. 138—139° (Found: C, 59·1; H, 6·1; N, 12·8. $C_{11}H_{14}O_3N_2$ requires C, 59·4; H, 6·3; N, 12·6%), N-*ethoxy-*, leaflets (from water), m. p. 166° (Found: C, 60·7; H, 7·0; N, 11·8. $C_{12}H_{16}O_3N_2$ requires C, 61·0; H, 6·8; N, 11·9%), and N-*benzyloxy-methylhippuramide*, leaflets (from alcohol), m. p. 133° (Found: C, 68·3; H, 6·1; N, 9·8. $C_{17}H_{18}O_3N_2$ requires C, 68·5; H, 6·0; N, 9·4%). *N*-Ethoxymethylbenzamide could not be isolated; it was rapidly transformed into methylenebisbenzamide in ethanolic 0·2N-hydrochloric acid. *N-n*-Butoxymethylbenzamide, isolated as a liquid, b. p. 180°/3 mm., was converted into *methylenebisbenzamide* during several weeks (Found: C, 69·3; H, 7·9; N, 7·2. $C_{12}H_{17}O_2N$ requires C, 69·6; H, 8·2; N, 6·8%). N-*Ethoxy-*, leaflets (from light petroleum), m. p. 55—56° (Found: C, 68·8; H, 7·6; N, 7·3. $C_{11}H_{15}O_2N$ requires C, 68·4; H, 7·3; N, 7·3%), and N-n-*butoxy-methylphenylacetamide* leaflets (from light petroleum), m. p. 56° (Found: C, 70·7; H, 8·4; N, 6·4. $C_{13}H_{19}O_2N$ requires C, 70·6; H, 8·6; N, 6·4%), were obtained normally.

*Ether of* N-*Benzoylserine Ethyl Ester and* N-*Hydroxymethylphenylacetylglycine amide* $[CH_2Ph{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH(NHBz){\cdot}CO_2Et]$.—*N*-Benzoylserine ethyl ester (Erlenmeyer, *Annalen*, 1904, **337**, 251) (1·0 g.), *N*-hydroxymethylphenylacetylglycine amide (0·8 g.), acetone (4·0 c.c.), and concentrated hydrochloric acid (0·05 c.c.) were warmed in a water-bath until solution was completed. By next morning a solid *ether* (0·43 g.) had crystallised. This was fractionally crystallised from aqueous alcohol and gave leaflets, m. p. 182—183° (Found: C, 62·7; H, 6·0. $C_{23}H_{27}O_6N_3$ requires C, 62·6; H, 6·1%).

*The ether of* N-*benzoylserine ethyl ester and* N-*hydroxymethylphenylacetamide*

$[CH_2Ph{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH(NHBz){\cdot}CO_2Et]$

prepared similarly, separated from dilute alcohol in needles, m. p. 110° (Found: C, 65·3; H, 6·3; N, 6·9. $C_{21}H_{24}O_5N_2$ requires C, 65·6; H, 6·3; N, 7·3%).

The foregoing ethers, like the parent hydroxy-compounds, when boiled with β-naphthol, ethanol, and hydrochloric acid under reflux for 20 minutes, gave the corresponding condensation product of β-naphthol.

N-*Benzyloxymethylphenylacetamide.* (*a*) This was prepared in the usual way. (*b*) *N*-Hydroxymethylphenylacetamide (3·0 g.) was heated for 6 hours 105—110° (oil-bath) with benzyl alcohol (10 c.c.) and 80% formic acid (0·4 c.c.). The excess of benzyl alcohol was removed under reduced pressure; the *product* separated from benzene in leaflets, m. p. 69—70° (Found: C, 75·0; H, 7·0; N, 5·5. $C_{16}H_{17}O_2N$ requires C, 75·3; H, 6·9; N, 5·5%). It was converted into the methylenebisamide by warming its solution in alcoholic hydrochloric acid for 20 minutes. It did not condense with β-naphthol in 14 days at room temperature in alcoholic N-hydrochloric acid.

*Sulphides.*—N-*Ethylthiomethylphenylacetylglycine amide.* Ethanethiol (5 c.c.), the *N*-hydroxymethylamide (1·5 g.), and concentrated hydrochloric acid (0·05 c.c.) were heated to 60—70° in a sealed tube for 15 minutes. The solid did not all dissolve and after being kept for 3 hours the mixture was filtered and the residue washed with ether. The *sulphide* crystallised from water in plates, m. p. 127° (Found: C, 58·9; H, 6·6; N, 10·7; S, 11·6. $C_{13}H_{18}O_2N_2S$ requires C, 58·6; H, 6·8; N, 10·5; S, 12·0%). It was treated with sodium hydroxide at room temperature and the liberated formaldehyde estimated: N-NaOH, 48 hours 0, 9 days 19%; 2N-NaOH, 48 hours, 15·1%. The compound is more stable than the *O*-ether.

N-*Ethylthiomethylphenylacetyl*-DL-*alanine amide* formed prisms (from dilute methanol), m. p. 124—125° (Found: C, 59·8; H, 7·3; N, 9·6; S, 11·3. $C_{14}H_{20}O_2N_2S$ requires C, 60·0; H, 7·1; N, 10·0; S, 11·4%). N-*Ethylthiomethylbenzamide*, formed prisms (from dilute methanol), m. p. 74—75° (Found: C, 61·6; H, 6·7; N, 7·2; S, 16·3. $C_{10}H_{13}ONS$ requires C, 61·6; H, 6·7; N, 7·2; S, 16·4%).

The sulphides condensed with β-naphthol to give 1-acylamidomethyl-2-naphthols after 3 hours' boiling with acidified ethanol.

*Formaldehyde and Benzylamine.*—Formaldehyde was condensed with benzylamine as described by Henry (*Bull. Acad. Roy. Belg.*, 1895, **29**, 23). The product distilled at 100°/0·005 mm., giving a pale yellow oil, which solidified and crystallised from aqueous ethanol in colourless prisms, m. p. 50° [Found: C, 80·7; H, 7·8; N, 11·7%; *M* (in camphor), 378. Calc. for $(C_8H_9N)_3$: C, 80·7; H, 7·6; N, 11·8%; *M*, 357]. This compound which was also prepared by Kempf's method (*loc. cit.*) was not stable in acid solution and the percentages of formaldehyde set free under two conditions were: (*a*) 2N-HCl added rapidly: 5 minutes, 18·3%; 1 hour, 27·4%. (*b*) 2 Equivs. added during 30 minutes: 59·5%. For comparison the corresponding figures for methylenebisdibenzylamine are (*a*) 1 hour, 20·5%, and (*b*) 86·4%.

*Condensation Products of Primary Amines and N-Hydroxymethylamides.—Bisbenzamidomethylmethylamine* (V; R = Ph, R′ = Me). *N*-Hydroxymethylbenzamide (5 g.) and a 23% solution of methylamine in glacial acetic acid (5 g.) were dissolved in glacial acetic acid (50 c.c.) and kept at 75° for 45 minutes, after which the solvent was removed under reduced pressure at 40°. Water (20 c.c.) was added to the residue and the insoluble benzamide (0·3 g.) removed. The filtrate was made alkaline to litmus with sodium hydroxide and left overnight at 0°. A colourless oil separated which was taken up in ethanol, and water was added until the mixture became turbid; crystals (1·0 g.; m. p. 120—124°) were gradually deposited; recrystallisation from dilute alcohol gave long prisms, m. p. 127—128° [Found: C, 68·8; H, 7·0; N, 14·1%; *M* (in camphor), 311. $C_{17}H_{19}O_2N_3$ requires C, 68·7; H, 6·4; N, 14·1%; *M*, 297]. The compound was soluble in acetone, alcohol, benzene, ether, ethyl acetate, or hot carbon tetrachloride, and insoluble in light petroleum and water. *Bisbenzamidomethyl*-n-*butylamine*, prepared (*a*) as above from *n*-butylamine and (*b*) by heating aqueous solutions of *n*-butylamine and *N*-hydroxymethylbenzamide on a water-bath for 12 hours, separated from alcohol in prisms, m. p. 115° (Found: C, 71·1; H, 7·5; N, 12·4. $C_{20}H_{25}O_2N_3$ requires C, 70·8; H, 7·4; N, 12·4%). This compound was more stable than methylenebisdibenzylamine. After it had been dissolved rapidly in 2N-hydrochloric acid no formaldehyde was found after 5 minutes; after 5 hours 16·5% of the total formaldehyde was split off. When 2 equivs. of hydrochloric acid were added during 30 minutes no formaldehyde was found in the solution.

*Methylbisphenylacetamidomethylamine*, prepared by the acetic acid method in low yield, separated from aqueous alcohol in prisms, m. p. 144° (Found: C, 70·3; H, 7·1; N, 13·0. $C_{19}H_{23}O_2N_3$ requires C, 70·2; H, 7·1; N, 12·9%). *Methyldi-*(β-*phenylpropionaminomethyl*)*amine*, prepared and crystallised similarly, formed plates, m. p. 118—119° (Found: C, 71·2; H, 7·6; N, 12·1. $C_{21}H_{27}O_2N_3$ requires C, 71·4; H, 7·7; N, 11·9%).

*n*-Butylamine did not react under either of the above conditions with *N*-hydroxymethylphenylacetamide or *N*-hydroxymethyl-β-phenylpropionamide.

*Reaction of* N-*Hydroxymethylamides and Secondary Amines.*—1-*Phenylacetylglycylamidomethylpiperidine*, $CH_2Ph{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}NC_5H_{10}$. (*a*) Aqueous formaldehyde (0·3 c.c; 40%) was slowly added to piperidine (0·3 c.c.) cooled in water. To this solution phenylacetyl-

glycine amide (0·5 g.) and alcohol (1·5 c.c.) were added and the mixture was boiled for 4 hours under reflux. (*b*) A solution of 1 : 1′-methylenebispiperidine (Braun and Röver, *Ber.*, 1903, **36**, 1196) and phenylacetylglycine amide in ethanol was heated for 4 hours. The crystals which separated on cooling were filtered off, washed with ether, and recrystallised from water in plates, m. p. 148° (Found : C, 66·1; H, 7·8; N, 14·5. $C_{16}H_{23}O_2N_3$ requires C, 66·4; H, 8·0; N, 14·5%). *Benzamidomethyldibenzylamine*, prepared as in (*a*), separated from alcohol in long prisms, m. p. 145—146° (Found : C, 80·1; H, 6·8; N, 8·9. $C_{22}H_{22}ON_2$ requires C, 80·0; H, 6·7; N, 8·5%). This compound did not liberate formaldehyde when mixed with hydrochloric acid at the room temperature under the conditions given for the condensation products with amides and primary amines. *Dibenzylphenylacetamidomethylamine* crystallised from alcohol in prisms, m. p. 99° (Found : N, 8·0. $C_{23}H_{24}ON_2$ requires N, 8·1%).

*Acetoxymethylamides.*—*N-Acetoxymethylphenylacetamide.* *N*-Hydroxymethylphenylacetamide (2 g.) was heated in glacial acetic acid (20 c.c.) at 75° for 30 minutes. The solvent was removed under reduced pressure at 40° and water added to the residue. The product (1·5 g.) which separated crystallised from benzene in needles, m. p. 98° [Found : C, 63·8; H, 6·4; N, 7·0%; *M* (in camphor), 198. $C_{11}H_{13}O_3N$ requires C, 63·8; H, 6·3; N, 6·8%; *M*, 207]. *N-Acetoxymethylphenylacetamide* in N-sodium hydroxide at room temperature split off 19·1% of the total formaldehyde in 48 hours and 22·3% in 9 days. The same compound in 2N-hydrochloric acid at room temperature split off the following percentages of formaldehyde : 20 hours, 13·8; 50 hours, 24·3; 112 hours, 44·8; 250 hours, 65·0; 16 days, 73·0. Only a trace of methylenebisamide was formed.

*N-Acetoxymethyl-β-phenylpropionamide* was prepared similarly from *N*-hydroxymethyl-β-phenylpropionamide and separated from benzene–*cyclo*hexane in prisms, m. p. 68—69° (Found : C, 65·5; H, 6·8; N, 6·5. $C_{12}H_{15}O_3N$ requires C, 65·2; H, 6·9; N, 6·3%).

*Model Peptides and their Attempted Condensation with Formaldehyde.*—1 : 4-Bishydroxymethyl-2 : 5-diketopiperazine was prepared by the action of formaldehyde on diketopiperazine at pH 8·5 (Cherbuliez and Feer, *loc. cit.*), but there was no condensation between diketopiperazine and formaldehyde at pH 0 or 3·6. The dihydroxy-compound (0·5 g.) and phenylacetamide (1·1 g.) were suspended in a buffer solution (15 c.c.) at pH 0 and warmed for a few minutes until solution was obtained. After a further 2 minutes a heavy white precipitate was obtained which was collected and washed with water. Crystallisation from hot ethanol yielded needles (0·85 g.), m. p. 210°, which did not depress the melting point of an authentic specimen of bisphenylacetamidomethane.

Phthaloylglycine chloride was prepared by a modification of the method described by Sheehan and Frank (*J. Amer. Chem. Soc.*, 1949, **71**, 1856). Phthaloylglycine (20 g.) (Gabriel, *Ber.*, 1907, **40**, 2648) and phosphorus pentachloride (20·5 g.) were suspended in dry carbon tetrachloride (150 c.c.) and the mixture heated, with shaking, on a water-bath until the evolution of hydrogen chloride ceased (about 20 minutes). A little undissolved material was filtered off and the carbon tetrachloride removed under reduced pressure. The oily residue soon crystallised on cooling, and recrystallisation from dry benzene gave needles (21 g.), m. p. 82—83° (Sheehan and Frank, *loc. cit.*, give m. p. 85°).

*Phthaloylglycine diethylamide.* Phthaloylglycine chloride (10·0 g.) was added, with shaking and cooling, during 10 minutes to a solution of diethylamine (25 c.c.) in dry carbon tetrachloride (75 c.c.). After 30 minutes at room temperature the heavy white precipitate was filtered off, washed with carbon tetrachloride and then with water, and dried. The *amide* crystallised from alcohol in needles (11 g.), m. p. 151° (Found : C, 64·3; H, 5·9; N, 10·4. $C_{14}H_{16}O_3N_2$ requires C, 64·6; H, 6·2; N, 10·7%), soluble in toluene or benzene, insoluble in light petroleum (b. p. 60—80°) or carbon tetrachloride.

*Phthaloylglycine morpholide*, prepared similarly in 85% yield, crystallised from water or alcohol in plates, m. p. 200—201° (Found : C, 61·2; H, 5·3; N, 10·4. $C_{14}H_{14}O_4N_2$ requires C, 61·3; H, 5·1; N, 10·2%), soluble in hot benzene, but insoluble in ligroin or carbon tetrachloride. For preparation of glycine diethylamide, phthaloylglycine diethylamide (4·6 g.) in suspension in 0·16N-alcoholic hydrazine hydrate (120 c.c.) was heated under reflux for 4 hours, the solvent evaporated under reduced pressure, and the syrupy residue heated with 2N-hydrochloric acid (50 c.c.) at 50° for 10 minutes. The phthaloylhydrazide (3·2 g.) was filtered off and the filtrate concentrated under reduced pressure. The *amide hydrochloride* separated and crystallised from hot alcohol as hygroscopic needles, m. p. 101° (Found : Cl, 21·7. $C_6H_{15}ON_2Cl$ requires Cl, 21·4%). *Glycine morpholide hydrochloride*, prepared similarly in 68% yield, crystallised from hot ethanol in hygroscopic needles, m. p. 106° (Found : Cl, 19·4. $C_6H_{13}O_2N_2Cl$ requires Cl, 19·7%).

*Phenylacetylglycine diethylamide.* Phenylacetyl chloride (2·1 c.c.) was added during 15 minutes to a cooled, stirred solution of glycine diethylamide hydrochloride (1·5 g.) in water (10 c.c.) to which was added magnesium oxide (1·5 g.). After 30 minutes at room temperature the mixture was made acid to Congo-red, and the solid collected, washed with dilute sodium hydroxide solution, and dried. The *phenylacetyl* derivative crystallised from ethanol as plates (1·4 g.), m. p. 84—85° (Found: C, 67·4; H, 8·0; N, 11·6. $C_{14}H_{20}O_2N_2$ requires C, 67·7; H, 8·1; N, 11·3%), soluble in hot benzene or chloroform. The *morpholide*, prepared similarly (75% yield), crystallised from ethanol in stout prisms, m. p. 175° (Found: C, 64·5; H, 6·9; N, 10·5. $C_{14}H_{18}O_3N_2$ requires C, 64·8; H, 6·9; N, 10·7%).

Phenylacetyl-DL-alanine was prepared by a modification of Shiple and Sherwin's method (*J. Biol. Chem.*, 1922, **53**, 472). Phenylacetyl chloride (7·7 c.c.) was added with vigorous stirring during 30 minutes to an ice-cold solution of DL-alanine (5·0 g.) in water (100 c.c.) in which was suspended magnesium oxide (6·0 g.). After a further 15 minutes' stirring the mixture was acidified and the precipitate filtered off, washed with water, and dried. The product was washed with benzene to remove phenylacetic acid and crystallised from hot ethanol, giving plates (10·2 g.), m. p. 150° (Shiple and Sherwin, *loc. cit.*, give m. p. 149—150°). Phenylacetylglycine ethyl ester, prepared (*a*) similarly from glycine ethyl ester hydrochloride or (*b*) by addition of pyridine to a chloroform solution of phenylacetyl chloride and glycine ethyl ester hydrochloride, crystallised from ethanol in needles, m. p. 82° (Found: N, 6·2. Calc. for $C_{12}H_{15}O_3N$: N, 6·3%). Hotter (*loc. cit.*) gives m. p. 79°. When the ester (1 g.) was boiled under reflux for 10 hours with morpholine (2·5 c.c.) and the excess of morpholine removed under reduced pressure only unchanged ester (0·9 g.), m. p. 81°, was obtained.

The following were also prepared: *Phthaloylglycine ethylamide*, colourless needles (from alcohol), m. p. 240° (Found: C, 62·2; H, 5·0; N, 12·0. $C_{12}H_{12}O_3N_2$ requires C, 62·1; H, 5·2; N, 12·1%). *Phthaloyl-DL-alanine morpholide*, colourless prisms (from alcohol), m. p. 172° (Found: C, 62·3; H, 5·5; N, 9·9. $C_{15}H_{16}O_4N_2$ requires C, 62·5; H, 5·6; N, 9·7%), and *diethylamide*, hexagonal prisms (from alcohol), m. p. 85° (Found: C, 65·5; H, 6·2; N, 10·2. $C_{15}H_{18}O_3N_2$ requires C, 65·7; H, 6·5; N, 10·2%). Glycine ethylamide hydrochloride, prisms (from ethanol), m. p. 128—130° (von Braun and Münch, *Ber.*, 1927, **60**, 350, give m. p. 134°) (Found: Cl, 26·0. Calc. for $C_4H_{11}ON_2Cl$: Cl, 25·6%). *DL-Alanine diethylamide hydrochloride*, needles (from ether–alcohol), m. p. 136° (Found: Cl, 20·5. $C_7H_{17}ON_2Cl$ requires Cl, 19·7%). *Phenylacetylglycine ethylamide*, plates (from aqueous methanol), m. p. 170° (Found: C, 65·2; H, 7·3; N, 12·5. $C_{12}H_{16}O_2N_2$ requires C, 65·5; H, 7·3; N, 12·7%). *Phenylacetyl-DL-alanine diethylamide*, prisms (from dilute methanol), m. p. 116° (Found: C, 68·7; H, 8·5; N, 10·3. $C_{15}H_{22}O_2N_2$ requires C, 68·7; H, 8·4; N, 10·7%). *Hippuroylmorpholide*, needles (from aqueous methanol), m. p. 150° (Found: C, 63·2; H, 6·6; N, 10·9. $C_{13}H_{16}O_3N_2$ requires C, 62·9; H, 6·5; N, 11·3%).

*Condensations with* p-*Cresol and Tyrosine.*—*3-Benzamidomethyl-*p-*cresol* (Me = 1). *N*-Hydroxymethylbenzamide (0·5 g.) and *p*-cresol (0·5 g.) in ethanol (2 c.c.) and concentrated hydrochloric acid (0·05 c.c.) were either (*a*) boiled under reflux for 20 minutes, or (*b*) dissolved and kept overnight. The *product* separated from alcohol in prisms, m. p. 160° (Found: C, 74·5; H, 6·0; N, 6·1. $C_{15}H_{15}O_2N$ requires C, 74·7; H, 6·2; N, 5·8%).

*3-Phenylacetylglycylamidomethyl-*p-*cresol*, similarly prepared, separated from alcohol as prisms, m. p. 154—155° (Found: C, 68·9; H, 6·3; N, 8·6. $C_{18}H_{20}O_3N_2$ requires C, 69·2; H, 6·4; N, 8·9%).

*3 : 5-Bishydroxymethyl-*N-*benzoyltyrosine.* *N*-Benzoyltyrosine (Erlenmeyer and Halsey, *Annalen*, 1899, **307**, 138) (1·0 g.), sodium hydroxide (0·2 g.), 40% formaldehyde (1·0 c.c.), and water (1·0 c.c.) were mixed and set aside for 14 days. The water was then evaporated *in vacuo* over sulphuric acid, leaving a sticky residue which was redissolved in a little water, filtered from a trace of solid, and made just acid to Congo-red. The precipitated *product* (0·6 g.) was crystallised several times from water, and gave colourless plates, m. p. 175—176° (decomp.) (Found: C, 62·8; H, 5·5; N, 4·2. $C_{18}H_{19}O_6N$ requires C, 62·6; H, 5·5; N, 4·1%).

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## 570. *The Action of Formaldehyde on Proteins. Part III.* The Hydrolysis of Formaldehyde-hardened Proteins by Enzymes.*

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It has been found that certain proteins treated with formaldehyde under acid conditions can be hydrolysed by papain and the products further hydrolysed by trypsin. From the hydrolysate from "Ardil" protein fibre, six formaldehyde-containing fragments have been isolated by adsorption on charcoal, Zeocarb-215, and cellulose, and analysed by paper chromatography. Acid hydrolysis has shown that aspartic and glutamic acid and at least three other amino-acids are associated with these six formaldehyde containing fragments. The results suggest (1) that *N*-methylenebisamide derivatives of aspartic and glutamic acid play a considerable part and (2) that lysine and arginine play little, if any, part in the reaction of proteins and formaldehyde under acid conditions.

In Part I (*J.*, 1950, 1493) of this series evidence was presented that under alkaline conditions formaldehyde acted on the amide groups in proteins and converted them into *N*-hydroxymethylamide groups. It was also noted that proteins treated with formaldehyde at pH 0 contained only a very low proportion of groups which reacted with 6-bromo-2-naphthol and it was regarded as probable that *N*-methylenebisamide groups were predominant, although cross linkages between amide and other groups were not excluded. Proteins insolubilised by formaldehyde under acidic conditions contain formaldehyde which falls into three different categories with regard to ease of removal. Some of the formaldehyde, including that combined in *N*-hydroxymethylamide groups, is removed by boiling water, part (including that united as *N*-methylenebisamide group) is split off by acids, and part is not recovered by acid hydrolysis but remains combined with tyrosine and possibly other amino-acids. While it is conceivable that regulated acid hydrolysis might degrade an insolubilised protein to more manageable fragments still containing methylene bridges it is certain that during the acid treatment some of the less stable methylene-bridged compounds would be broken down, and that new compounds would be formed to an extent depending on the conditions of hydrolysis. Preliminary experiments indicated that alkaline hydrolysis presented similar difficulties and in these circumstances recourse was had to enzymic hydrolysis. It was found that ground-nut protein, soya-bean protein, and casein, after acid-insolubilising treatment, were not attacked by papain, pepsin, or trypsin, but when the loosely bound formaldehyde was removed by boiling water the remaining protein was attacked by papain, particularly if the enzyme was activated by hydrogen cyanide at pH 8—9. Hydrogen sulphide, sodium hydrogen sulphite, and cysteine were much less effective than hydrogen cyanide. The hydrolyses were followed by formaldehyde titration and the results are summarised in Table 1. The percentage of peptide groups hydrolysed was calculated by using the value 0·007 mole of amino-group per g. of protein derived from Chibnall's analytical figures (Traill, *J. Soc. Dyers Col.*, 1945, **61**, 150); the acid hydrolysis of ground-nut protein gave a Sörensen figure of 0·0066 mole per g. of protein, in reasonable agreement with the above. The high formaldehyde content of the proteins treated with formaldehyde under alkaline conditions is probably due to reaction with arginine, as it is found that arginine, ground-nut protein, and acid–formaldehyde-insolubilised ground-nut protein after treatment with alkaline formaldehyde no longer respond to the Sakaguchi test for arginine.

After these preliminary experiments attention was concentrated upon "Ardil" fibre made by Imperial Chemical Industries Limited by the action of formaldehyde on ground-nut protein (Traill, *loc. cit.*), and Table 2 summarises the results obtained when the fibre was boiled with water to remove the labile formaldehyde and then digested with cyanide-activated papain.

* Part II, preceding paper.

TABLE 1. *Papain digestion of formaldehyde-treated proteins after removal of labile formaldehyde.*

Protein (1·0 g.) suspended in water (50 c.c.) containing papain (0·5 g.) and potassium cyanide (0·2 g.).

| Protein | $CH_2O$ treatment | $CH_2O$ (%) left in protein after water-treatment | Time of digestion (days) | Sörensen titration; 25 c.c. of solution, 0·05N-NaOH | Sörensen titration, control | Hydrolysis (%) |
|---|---|---|---|---|---|---|
| Ground-nut ... | none | none | 0·25 | 16·2 | 1·2 | 22 |
| ,, ... | pH 0 | 1·6 | 3 | 15·5 | ,, | 21 |
| ,, ... | pH 3 | 0·9 | ,, | 14·0 | 1·3 | 20 |
| ,, ... | 5% $K_2CO_3$ | 2·0 | ,, | 18·1 | 1·2 | 26 |
| ,, ... | 10% $K_2CO_3$ | 2·8 | ,, | 16·1 | ,, | 22 |
| Casein ..... | none | none | 0·25 | 16·5 | ,, | 22 |
| ,, ..... | pH 0 | 1·7 | 3 | 16·2 | ,, | 22 |
| Soya bean ..... | none | none | 0·25 | 17·1 | ,, | |
| ,, ..... | pH 0 | 1·8 | 3 | 16·9 | ,, | |

TABLE 2. *Hydrolysis of " Ardil " fibre with cyanide-activated papain at* 40°.

The fibre (1 g.) was suspended in water (50 c.c.) containing papain (0·5 g.) and potassium cyanide (0·2 g.).

| Time of digestion (days) | Undissolved protein (g.) | Sörensen titration of solution | Sörensen titration of control | Amino-groups set free (mole per 1 g. of protein) | Hydrolysis of peptide links (%) |
|---|---|---|---|---|---|
| 1·5 | 0·15 | 3·3 | 0·4 | 0·0004 | 9·5 |
| 3·25 | 0·05 | 6·2 | 0·9 | 0·0015 | 20 |
| 4·5 | ,, | 8·1 | ,, | 0·0018 | 24 |
| 5·5 | ,, | 8·2 | ,, | 0·0018 | 24 |
| 7·5 | ,, | 8·3 | ,, | 0·0019 | 26 |

It was possible that the insolubilising process might have produced a non-homogeneous material with a higher formaldehyde content on the outer layer of the fibre than in the core. In order to test this the formaldehyde content of the original fibres and of the residues obtained after varying periods of digestion were determined. The results summarised in Table 3 indicate that the fibre is homogeneous after being insolubilised. By using

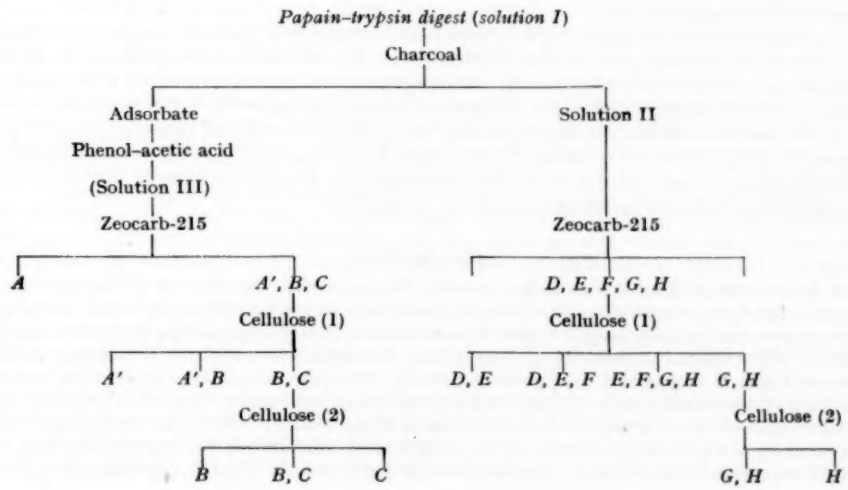
TABLE 3. *Formaldehyde in undissolved fractions of " Ardil " fibres after papain digestion.*

| Time of digestion (hours) | Ardil undissolved (g.) | $CH_2O$ in undissolved material (%) |
|---|---|---|
| Control fibre | 5·0 | 1·2, 1·2 |
| 4 | 3·1 | 1·1, 1·2 |
| 6 | 2·6 | 1·2, 1·2 |
| 9 | 1·3 | 1·1, 1·1 |
| 10 | 1·1 | 1·2, 1·3 |

the same sample of fibre it was found that the degree of hydrolysis after 4—5 days' digestion varied from 25% to 40% with different samples of commercial papain. The degree of hydrolysis, 25%, obtained with papain indicated that the average molecular size of the polypeptide in the digest solution corresponded to four amino-acids and as the object of this work was to obtain the methylene-bridged amino-acids corresponding to the methylene bridges in the protein it was decided to hydrolyse further the papain digest solution. Although pepsin and trypsin did not attack the water-boiled insolubilised protein, it was found that they brought about a further hydrolysis of the papain digest solution. Trypsin gave the better result and took the hydrolysis to 70—80%. The resulting solution when tested with dimedone showed no free formaldehyde, and after acid hydrolysis and distillation the formaldehyde recovered corresponded to 95% of the formaldehyde taken up by the fibre. It is improbable that the formaldehyde has been liberated during the digestion and has subsequently combined with amino-acids present, because the addition of free formaldehyde to a digest solution stopped the hydrolysis. We consider that the digest solution contains at this stage not only the methylene-linked polypeptides and amino-acids corresponding to the methylene bridges in the original insolubilised proteins, but also formaldehyde-free amino-acids and polypeptides. For the immediate object of this work, only the

compounds containing methylene bridges were of interest, and attention was focused on those compounds from which formaldehyde was liberated by acid hydrolysis; compounds containing formaldehyde linked to aromatic nuclei are not considered at this stage. The further hydrolysis by trypsin of the papain digest solution gave variable results depending on the nature of the fibre, the extent to which the papain digestion had been carried, and the previous history of the trypsin. The results described in this paper were obtained with selected digest solutions in which the total hydrolysis by the two enzymes had proceeded to 70—80%. When the hydrolysis was stopped at an earlier stage the polypeptides obtained were naturally different, but two-dimensional paper chromatograms showed similar characteristics. The less hydrolysed solutions gave diffuse areas which on further hydrolysis could be resolved into spots of increasing definition.

The papain–trypsin digest (solution I) obtained in the manner described above gave twenty-four sharply defined spots on a two-dimensional paper chromatogram and several diffuse areas of colour, after development with ninhydrin. Solution I was treated with charcoal and filtered, and the adsorbate was eluted with a solution of phenol and acetic acid to give solution III. A sample was evaporated to dryness under reduced pressure. The solid residue contained 7—9% of formaldehyde determined by acid hydrolysis; the amount of formaldehyde in the charcoal adsorbate was less when the hydrolysis had not been carried so far. As " Ardil " fibre, after being boiled with water, contained 1·7% of formaldehyde (Tetlow, *J. Sci. Food Agric.*, 1950, **1**, 193) a considerable concentration of hydrolysable methylene-bridged material had been effected. Solution III gave three ninhydrin-reacting spots *A*, *B*, *C* on a two-dimensional paper chromatogram; *A* was subsequently split into *A* and a similar material *A'* by the use of cellulose columns. All four compounds contained formaldehyde which was set free by acid hydrolysis. The filtrate from the charcoal, solution II, was separated by a column of Zeocarb-215 into a number of fractions from which only one group contained acid-hydrolysable formaldehyde. This group of fractions was mixed and a sample on a two-dimensional paper chromatogram gave five ninhydrin-reacting spots, *D*—*H*. The solution of these substances was evaporated to dryness under reduced pressure, dissolved in butanol–acetic acid–water, and put through a cellulose column. The fractions were examined on two-dimensional paper chromatograms which showed a partial separation into *D* and *E*; *D*, *E*, and *F*; *E*, *F*, *G*, and *H*; and *G* and *H*; but only the fractions containing *G* and *H* liberated formaldehyde on acid hydrolysis. The solution of *G* and *H* was evaporated to dryness, dissolved in butanol–diethylamine–water, and put through a cellulose column, and *H* was isolated. The process is represented in the annexed scheme.

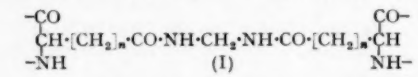


The substances *A*, *A'*, *B*, *C*, and *H*, and the mixture of *G* and *H*, were hydrolysed by acid, and the hydrolysates analysed by two-dimensional paper chromatograms; two chromatograms were made in each case, with phenol and butanol–acetic acid, and phenol and butanol–propanol–diethylamine as the pairs of solvents. The results are summarised in Table 4.

TABLE 4.

| Acid | *A* | *A'* | *B* | *C* | *H* | *G–H* |
|---|---|---|---|---|---|---|
| Aspartic | + | + | + | + | + | + |
| Glutamic | + | + | + | + | + | + |
| Leucine | + | + | | + | + | + |
| Valine | + | | + | + | | + |
| Alanine | + | + | | | + | + |
| Serine | | + | + | + | + | + |
| Proline | | + | + | + | | |
| Tyrosine | | | + | + | | |
| Phenylalanine | | | + | + | | |
| Cystine | | | | | | + |

The results described represent the first step towards the isolation of the methylene-bridged units present in insolubilised ground-nut protein. Numerous suggestions have been made concerning the nature of these bonds (French and Edsall, *Adv. Protein Chem.*, 1945, **2**, 305; Traill, *Chem. and Ind.*, 1950, 23), but the absence of lysine and arginine from the formaldehyde-containing fragments, also observed by Waldschmidt-Leitz (*Zellwolle, Kunstseide, Seide*, 1941, **46**, 444) for hardened casein, indicates that the frequent suggestion that these amino-acids play an important part in acid–formaldehyde reactions is not in agreement with these experimental observations. On the other hand all the formaldehyde-containing fragments yield the dibasic, aspartic and glutamic acid together with at least three other amino-acids, and the suggestion that the amide groups of asparagine and glutamine participate in the insolubilising process is strongly supported by our observations. A methylenebisamide structure of type (I) would probably be readily formed under the



(I)

acid-insolubilising conditions and a considerable degree of stability towards acids would be expected by analogy with the properties of model compounds (see Part I). Such a structure would also be consistent with the observations that the acid-hydrolysable formaldehyde content and the amide value of substance *A* were in the ratio of 1 : 1·8, but in other fractions it is possible that serine-*O*- or cysteine-*S*-derivatives of *N*-hydroxymethylamide compounds also contribute.

Further evidence in support of the view that the amide groups are largely concerned in the formation of methylene bridges comes from the action of formaldehyde in alkaline solution on acid-insolubilised proteins. When ground-nut protein is treated with formaldehyde in 10% potassium carbonate solution and then condensed with 6-bromo-2-naphthol, 2·5% of bromine is taken up, corresponding to combination with 35 amide groups/$10^5$ g. of protein or 27% of the total amide groups (Part I). If the protein is first insolubilised in acid solution and then submitted to this treatment the bromine uptake is only 0·5—0·6% corresponding to seven amide groups/$10^5$ g. of protein.

## EXPERIMENTAL

*Papain Hydrolysis of Formaldehyde-treated Proteins.*—Samples (10—15 g.) of ground-nut protein, casein, and soya-bean protein were treated with formaldehyde at pH 0 and, in the case of the first-named protein, at pH 3, and in presence of 5 and 10% potassium carbonate solution (Part I). The treated protein (10 g.) was heated with water (500 c.c.) for 30 minutes at 100°. The water was decanted and the process repeated. The protein was then filtered off, washed, and dried at 100° for 2 hours. Papain (0·5 g.) was mixed with water (30 c.c.), left for 0·5 hour, and then filtered off. The filtrate was made up to 50 c.c., the formaldehyde-treated and boiled protein (1 g.) and potassium cyanide (0·2 g.) were added, and the mixture was incubated at 40°. The pH was initially 8—9 and at the end of digestion about 8. Control experiments with the

enzyme alone were carried out at the same concentration. Samples (10 c.c.) were withdrawn at the intervals stated in Table 1 and titrated by the Sörensen method.

*Enzymic Hydrolysis of "Ardil" Fibre.*—The fibre was supplied by Imperial Chemical Industries Limited, Nobel Division, and had been prepared as described by Traill (*J. Soc. Dyers Col.*, 1945, **61**, 150). The formaldehyde content was 2·8%. "Ardil" fibre (50 g.) was heated with water as described above and dried; the formaldehyde content was then 1·6%. Papain (2·5 g.) was extracted with water (150 c.c.), and the filtrate made up to 250 c.c. 25 C.c. of this solution gave a Sörensen titration of 1·1 c.c. with 0·05N-sodium hydroxide. Boiled "Ardil" fibre (5·0 g.) and potassium cyanide (2·0 g.) were added to the papain solution (250 c.c.), and the mixture was incubated at 40°. The pH at the start was 8·6. After 7 days the protein solution, was filtered from the insoluble residue (0·1 g.); 25 c.c. of this solution gave a Sörensen titration of 15·1 c.c. (0·1N-sodium hydroxide), and the control 0·8 c.c. To the light brown solution, 15% trichloroacetic acid solution (80 c.c.) was added and the precipitate (0·9 g.; formaldehyde, 0·05%) collected. The excess of trichloroacetic acid was extracted with ether (3 × 120 c.c.), and the solution then aerated for 30 minutes to remove ether; the volume obtained was 320 c.c., and 10 c.c. gave a Sörensen titration of 5·6 c.c. (0·1N-sodium hydroxide). The solution was adjusted to pH 8·5 by addition of 2N-sodium hydroxide (about 8·0 c.c.), and trypsin (2 g.) added; 10 c.c. of this solution then gave a Sörensen titration of 6·6 c.c. (0·1N-sodium hydroxide). Thymol was added as a preservative and the solution incubated for 5 days at 40°, 2N-sodium hydroxide solution being added from time to time to keep the solution just alkaline to phenolphthalein. Buffered solutions were not used because of the subsequent difficulties in chromatography.

| Time of digestion (days) | 0 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Sörensen titration of 10 c.c.; c.c. 0·1N-NaOH | 6·6 | 8·8 | 9·2 | 9·8 | 9·8 | 9·8 |
| Sörensen titration of 10 c.c. of control papain–trypsin solution | 1·1 | 1·2 | 1·3 | 1·4 | 1·4 | 1·4 |

A sample of this solution was made faintly acid with acetic acid and mixed with dimedone solution. No precipitate was formed overnight, showing the absence of any quantity of formaldehyde. Another sample of the solution was hydrolysed in 6N-sulphuric acid, and the formaldehyde formed distilled into a solution of dimedone. The weight of the precipitate corresponded, to formaldehyde (55 mg.) in the total digest solution. "Ardil" fibre (5 g.), hydrolysed similarly gave a precipitate corresponding to 60 mg. of formaldehyde.

*Charcoal Adsorption.*—Charcoal (30 g.) was stirred for 1 hour with 5% acetic acid, filtered off, and washed with distilled water. The wet charcoal was then added to the digest solution (I) (620 c.c.) prepared from "Ardil" fibre (10 g.), and the mixture stirred for 1 hour. The charcoal was then filtered from the pale yellow solution (II), and stirred for 1 hour with a solution of phenol (20 g.) in 20% acetic acid (400 c.c.). The charcoal was collected and the filtrate concentrated at reduced pressure and below 50° to a thick syrup. Addition of ether or ethanol precipitated a pale yellow amorphous powder (III) which was washed with ether and dried (yield, 0·8 g.; formaldehyde content, 8·2%). A two-dimensional paper chromatogram by the descending method (Consden, Gordon, and Martin, *Biochem. J.*, 1944, **38**, 224), with (i) butanol–acetic acid (Partridge, *Biochem. J.*, 1948, **42**, 238) and (ii) 80% phenol or 80% phenol containing 1% of ammonia gave, on development with ninhydrin at 100°, three spots, *A*, *B*, and *C*. $R_F$ values in the order of the solvents are: *A*, purple, 0·13, 0·46, 0·76; *B*, blue, 0·44, 0·64, 0·56; *C*, purple, 0·58, 0·86, 0·78. In subsequent experiments a solvent was used containing *n*-butanol (70 c.c.), *n*-propanol (28 c.c.), water (25 c.c.), and diethylamine (2 c.c.), instead of phenol–ammonia.

*Fractionation on Zeocarb-215.*—A Zeocarb-215 column (15 × 2·5 cm.) containing Zeocarb (15 g.; 60—80 B.S.S. mesh) was prepared as described by Partridge and Westall (*Biochem. J.*, 1949, **44**, 418). An aqueous solution of the mixed peptides (III) (1·0 g.) was run on to this column and, after being washed with water until the washings were neutral, the peptides were eluted with 0·15N-ammonia until the ammonia began to come through the column. The eluate was collected in 45 fractions (each 15 c.c.). Fractions 1—20 inclusive and 25 and 26 contained no substances reacting with ninhydrin. Fractions 21—24 were each put on a one-dimensional paper chromatogram with 80% phenol as solvent. Fraction 21 contained apparently a single peptide (*A*) and fractions 22—24 mixtures of *A*, *B*, and *C*. 1 C.c. of *A* was hydrolysed with 1 c.c. of concentrated hydrochloric acid under reflux for 40 hours. The bulk of the hydrochloric acid was then removed by evaporation under reduced pressure and the volume made up to 1·0 c.c. with distilled water. Two-dimensional chromatograms, using 80% phenol, butanol–acetic acid, and butanol–propanol–diethylamine, showed the presence of five amino-acids in the hydrolysate. The $R_F$ values, in the order of the solvents, are: aspartic acid, weak spot, 0·18,

0·22, 0·04; glutamic acid, very strong spot, 0·31, 0·25, 0·05; alanine, weak spot, 0·56, 0·30, 0·20; valine, weak spot, 0·82, 0·49, 0·40; leucine, strong spot, 0·88, 0·67, 0·54. These $R_F$ values are within ±0·02 of the values found for the pure amino-acids singly. 7 C.c. of fraction 21 * after hydrolysis with 6N-sulphuric acid and distillation into potassium cyanide (Walker, " Formaldehyde," Reinhold Publ. Corp., p. 263) gave 9·5 mg. of formaldehyde. Determination of ammonia by hydrolysis (Wormall and Kaye, *J. Soc. Chem. Ind.*, 1945, **64**, 75) gave 17·5 mg. of amide group in 7·0 c.c. of the same solution.

*Fractionation on a Cellulose Column.*—A column (20·0 × 2·0 cm.) of powdered cellulose (10 g.; Whatman, Ashless) was prepared with the butanol–acetic acid mixture. The mixed peptides III (0·5 g.) dissolved in the butanol–acetic acid mixture were brought on to the column which was then extracted with the same solvent and the eluate collected in 20 fractions (each 10 c.c.). One-dimensional paper chromatograms with 80% phenol on each fraction showed that fractions 4 and 5 contained peptides, *B* and *C* fractions 6 contained *B* and a peptide *A'*, resembling *A*, and fractions 7—11 contained *A'*. The other fractions contained no peptides or amino-acids. All peptide-containing fractions also contained formaldehyde, detected by hydrolysis with sulphuric acid (1·5 c.c. of 9N to 1·0 c.c. of solution), distillation, and addition of chromotropic acid (McFadyen, *J. Biol. Chem.*, 1945, **158**, 107). Fraction 10 was evaporated to dryness under reduced pressure and hydrolysed with 5N-hydrochloric acid. Two-dimensional paper chromatograms of the hydrolysate showed the presence of six amino-acids, whose $R_F$ values for the same solvents as before and in the same order were: aspartic acid, 0·16, 0·18, 0·04; glutamic acid, 0·32, 0·27, 0·06; serine, 0·34, 0·20, 0·13; alanine, 0·56, 0·29, 0·20; proline, 0·90, 0·32, 0·22; leucine, 0·88, 0·68, 0·54. Fractions 4 and 5, containing the mixed peptides *B* and *C*, were evaporated to dryness and the solid (80 mg.) was dissolved in a small volume of the butanol–propanol–diethylamine mixture. This solution was brought on to a cellulose column (10 g.; Whatman, Ashless), prepared with the above solvent, and then developed with the same solvent. The eluate was collected in 20 fractions (each 4 c.c.). Fractions 8 and 9 contained peptide *C* only, 10 and 11 contained *B* and *C*, and 12—15 *B* only. The other fractions contained no substances reacting with ninhydrin. Peptides *B* and *C* both contained formaldehyde liberated on hydrolysis with acid. Fraction 8, peptide *C*, was evaporated to dryness and the residue (9 mg.) hydrolysed with 5N-hydrochloric acid. Two-dimensional paper chromatograms with the same solvents as before showed the presence of eight amino-acids: aspartic acid, $R_F$ 0·17, 0·18, 0·13; glutamic acid, 0·33, 0·26, 0·05; serine, 0·34, 0·19, 0·13; proline, 0·91, 0·32, 0·23; tyrosine, 0·56, 0·43; valine, 0·79, 0·48, 0·40; leucine, 0·84, 0·66, 0·54; phenylalanine, 0·85, 0·62, 0·56. Fraction 13, peptide *B*, was evaporated to dryness and the residue (12 mg.) hydrolysed. Two-dimensional paper chromatograms showed the presence of seven amino-acids, the same solvents as before being employed: aspartic acid, $R_F$ 0·18, 0·19, 0·05; glutamic acid, 0·32, 0·26, 0·07; serine, 0·34, 0·20, 0·14; proline, 0·90, 0·32, 0·24; tyrosine, 0·57, 0·44, 0·26; valine, 0·77, 0·50, 0·41; phenylalanine, 0·84, 0·62, 0·58.

*Solution II, the Charcoal Filtrate.—Fractionation on Zeocarb-215.* The column was constructed in three sections: (*a*) 15 × 2·5 cm. (15 g.), (*b*) 10 × 1·8 cm. (10 g.), and (*c*) 6 × 1·5 cm. (7·5 g.) (Partridge, *loc. cit.*). The charcoal filtrate, solution II, was run through the column, and the peptides and amino-acids eluted with 0·15N-ammonia, and collected in forty fractions (each 25 c.c.). Fractions 21—25 contained acid-hydrolysable formaldehyde, and fractions 2—32 contained substances reacting with ninhydrin. Fractions 21—25 were mixed and evaporated to dryness under reduced pressure below 50°. Two-dimensional paper chromatograms with 80% phenol and butanol–propanol–diethylamine gave five spots: D, $R_F$ 0·63, 0·21; E, 0·49, 0·31; F, 0·28, 0·50; G, 0·26, 0·66; H, 0·18, 0·70.

*Fractionation on cellulose.* A column of powdered cellulose (15 × 7 cm.; Whatman, Ashless) was prepared with butanol–acetic acid. The mixed peptides *D*—*H* were dissolved in the same solvent (15 c.c.) and put on the column, which was eluted with butanol–acetic acid, and the eluate collected in 35 fractions (each 15 c.c.). Fractions 17—33 contained peptides or amino-acids, fractions 23—33 contained acid-hydrolysable formaldehyde, fractions 17—20 contained *D* and *E*, fraction 21 *D*, *E*, and *F*, fraction 22 *E*, *F*, *G*, and *H*, and fractions 23—33 *G* and *H*. Fractions 23—33 were mixed, evaporated to dryness under reduced pressure and the solid dissolved in butanol–propanol–diethylamine. This solution was put on a cellulose column (10 g.) which was then developed with the same solvent, and the eluate collected in twenty-five fractions (each 15 c.c.); fractions 3—6 contained *G* and *H* with traces of *D*, *E*, and *F*; fractions 7—13 contained *G* and *H*; and fractions 15—23 *H* only. All fractions contained acid-hydrolysable

* In the case of polypeptides other than *A*, mentioned in Table 4, formaldehyde was detected qualitatively only; quantitative work is in progress.

formaldehyde. The last-mentioned fractions 15—23 were evaporated to dryness under reduced pressure and the solid hydrolysed with 5N-hydrochloric acid (5 c.c.) for 30 hours. After removal of the hydrochloric acid by evaporation under reduced pressure the residue was dissolved in water (2 c.c.) and put on two-dimensional paper chromatograms with phenol, butanol–acetic acid, and butanol–propanol–diethylamine as solvents. The amino-acids found, with $R_F$ values in the order of the solvents were: aspartic, 0·17, 0·18, 0·04, weak; glutamic, 0·32, 0·28, 0·05, strong; serine, 0·35, 0·20, 0·12, very strong; alanine, 0·54, 0·29, 0·20, strong; leucine, 0·85, 0·67, 0·54, strong.

Our thanks are offered to Drs. S. M. Partridge and G. R. Tristram for many helpful discussions and to Imperial Chemical Industries Limited, Nobel Division, for their " Ardil " fibre.

THE UNIVERSITY, SHEFFIELD, 10. [*Received, April 2nd,* 1952.]

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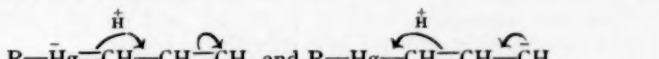
## 571. *The Preparation of Some Unsaturated Aliphatic Derivatives of Mercury and Boron.*

By EUGENE ROTHSTEIN AND ROWLAND W. SAVILLE.

Preparation of certain unsaturated aliphatic derivatives of mercury and boron is described. Most of these substances can be obtained in small yield only and are very unstable: they tend to decompose either spontaneously or in moist air. Mercury derivatives often afford the metal whereas the oxide is the main product in the case of boron compounds.

WHEN in their combined states mercury and boron have four and six electrons respectively in their valency shells; they present examples of non-polar atoms which could perhaps co-ordinate electrons from an attached unsaturated system in order to occupy the remaining orbitals, affording structures such as



The stable complexes which borines and boron halides form with electron-donating groups conform with this view, as does the instability of diallylmercury, particularly when freed from ether (Vijayaraghavan, *J. Indian Chem. Soc.*, 1940, **17**, 589; 1943, **20**, 318), which quite possibly may arise from the same cause:

In this case, however, the high resonance energy of the allyl *radical* might well lead to a considerable diminution of the dissociation energy of the carbon–mercury linkage.

Results of exploratory work on the synthesis of aliphatic allyl derivatives of the two elements discussed in this paper were disappointing: the mercury derivatives were found to be extremely unstable, and it was not possible to prepare the required dialkylallyl borines, $CH_2{:}CH{\cdot}CH_2{\cdot}BR_2$. The difficulty lies in the instability of even the saturated mixed alkyl derivatives of the two elements; indeed, only those of mercury have been isolated.

*Mercury Derivatives.*—Except for diallylmercury (Kharasch and Swartz, *J. Org. Chem.*, 1939, **3**, 405; Vijayaraghavan, *loc. cit.*) and analogues from a few halogen derivatives of ethylene and acetylene, unsaturated aliphatic derivatives of mercury HgRR′ are unknown. Kharasch and Swartz however have prepared allylphenyl- and allylbenzyl-mercury by interaction of allylmagnesium bromide and the appropriate mercuric chloride, R·HgCl. Allylethylmercury, now prepared in a similar way, decomposes when distilled or, rather more slowly, at room temperature.

The reverse reaction of ethylmagnesium iodide with allylmercuric iodide failed to yield allylethylmercury, the products being metallic mercury, diethylmercury, and ethylmercuric

iodide. Diallyl in this case could not be isolated, but Oppenheim (*Ber.*, 1871, **4**, 671) using diethylzinc in a similar reaction was able to do so. The presence of mercury amongst the decomposition products suggests the intermediate formation of diallylmercury. This follows because ethylphenylmercury, for instance, which is known to decompose slowly yielding a mixture of diethyl- and diphenyl-mercury only (Hilpert and Grüttner, *Ber.*, 1951, **84**, 906) does not eliminate mercury. The latter, if a product of the reaction, is usually the result of the further decomposition of the diallylmercury.

The isomerisation of the allyl derivatives to prop-1-enyl derivatives failed; mild conditions left the starting material unchanged; more drastic reagents (*e.g.*, potassium *tert.*-butoxide) yielded mixtures including diallylmercury, the presence of which was inferred by its conversion into allylmercuric bromide by ethereal bromine (Vijayaraghavan, *loc. cit.*). Finally, the prop-1-enyl derivative could not be obtained by partial reduction of prop-1-ynylmercuric chloride or of diprop-1-ynylmercury. Either the molecule underwent disruption or poisoning of the catalyst occurred.

*Boron Derivatives.*—As noted above, mixed aliphatic borines $BR_2R'$ have not been prepared. 2-Chlorovinylboron dichloride $Cl{\cdot}CH{:}CH{\cdot}BCl_2$ results from condensation of acetylene and boron trichloride and can be hydrolysed to the corresponding acid (Arnold, U.S.P. 2,402,509, 2,402,589/1946), whilst tri-*n*-butylborine affords, among other products, *n*-butyl dibutenylborate when halogenated with *tert.*-butyl hypochlorite (Johnson, Snyder, and Van Campen, *J. Amer. Chem. Soc.*, 1938, **60**, 115). The only other unsaturated organoboron compound, though not recognised as such, which has been isolated is the product of the reaction between diethyl zinc and triallyl borate (Councler, *J. pr. Chem.*, 1878, **18**, 371). The materials employed were evidently impure and in only one experiment was a zinc-free liquid, b. p. 110—112°, isolated. In the absence of a boron analysis, the analytical figures (C and H), which were not consistent with those required for triallylborine, appeared to fit the glyceride $C_3H_5B$. Repetition of the experiment afforded a product, b. p. 115—120°, the boron analysis of which corresponds to that of ethyl diallylborate (requires B, 7·9%) and not to the oxygen-free glyceride (requires B, 21·2%). Councler's experiments were carried out in the absence of a solvent; when the reaction occurred in tetralin the products were an unidentified unsaturated ester, b. p. 85—95°, and the allyl diethylborate. The methallyl ester was similarly obtained from trimethallyl borate.

There is a general tendency for reactions which are designed to yield mixed borines, *e.g.*, $BR_2R'$, to give a mixture of the symmetrical compounds $BR_3$ and $BR'_3$ (cf. Krause, *Ber.*, 1931, **64**, 2112) and this suggested that either allyldibutylborine or a mixture of triallyl- and tributyl-borine should result from the reaction between di-*n*-butylboron bromide and allylmagnesium bromide. However, in this case the products isolated were tri-*n*-butylborine and bisdiallylboron oxide $[(C_3H_5)_2B]_2O$. The latter like other derivatives of diallylboric acid is thermally stable if distilled in oxygen-free nitrogen but slowly decomposes to boric acid in air. Bisdiallylboron oxide was also prepared from boron trifluoride and allylmagnesium bromide, and the methyl ester resulted from the reaction between the Grignard compound and methyl borate. The preparation of prop-1-enylboric acid by elimination of methanol from di-*n*-butyl 3-methoxypropylborate was unsuccessful. The yields of the unsaturated boron compounds were very low and varying amounts of boric acid were always obtained. All aliphatic boron derivatives are very sensitive to aerial oxidation and this may explain why only small amounts could be isolated.

## Experimental

### *Mercury compounds.*

*Attempted Preparation of Prop-1-enylmercury Derivatives.*—Allylmercuric iodide was deposited unchanged when its solution in hot aqueous sodium hydroxide cooled. The substance is stable to cold concentrated hydrochloric acid but the liquid evolves an unsaturated gas, probably propylene, when heated. There was no condensation when the mercury compound was mixed with freshly distilled benzaldehyde and a drop of piperidine. Warming it with potassium *tert.*-butoxide in *tert.*-butyl alcohol yielded a dark grey solution with the characteristic odour of diallylmercury. The addition of ethereal bromine precipitated allylmercuric bromide (m. p. 126°

after crystallisation from alcohol) from the filtered solution. Metallic mercury and unchanged allylmercuric iodide were amongst the other products of the reaction.

*Derivatives of Propyne* (cf. Hurd, Meinert, and Spence, *J. Amer. Chem. Soc.*, 1930, **52**, 1138; Heilbron, Jones, and Weedon, *J.*, 1945, 81).—Purified acetylene was passed for 2 hours into a solution of sodamide, prepared from sodium (35 g.) and liquid ammonia (500 c.c.), at a rate of 3 l./min. and the sodium acetylide converted into propyne by addition of methyl iodide (210 g.), the mixture being kept at −40°. The gas was purified by passage through water, 10% sulphuric acid and a calcium chloride tower, Bunsen non-return valves being fitted between the various parts of the apparatus, and finally led into a vigorously stirred solution of ethylmagnesium bromide prepared from ethyl bromide (109 g., 1 mol.). The latter was contained in a 1½-l. flask fitted with a gas-inlet tube, a mercury-seal stirrer, and a liquid-ammonia condenser (Vaughn and Poggi, *J. Chem. Educ.*, 1931, **8**, 2433), the whole being cooled to −40°. The ammonia condenser was replaced by one of the ordinary type and the liquid allowed gradually to warm to room temperature. Mercuric chloride (160 g.) was then added in small portions, and the product isolated in the usual manner. This material was extracted, by means of a Soxhlet extractor, with acetone (3 × 100 c.c.). *Prop-1-ynylmercuric chloride* separated from the acetone in glistening plates and recrystallised from methyl alcohol as feathery plates, m. p. 135—136° (Found: Cl, 13·7; Hg, 73·1. $C_3H_3ClHg$ requires Cl, 12·9; Hg, 72·9%). Owing to the high mercury content it was impossible to obtain satisfactory analysis figures for carbon and hydrogen. Treatment with potassium cyanide solution afforded dipropynylmercury, separating from methyl alcohol or toluene in colourless glistening plates, m. p. 203—204° (Found: C, 26·5; H, 2·4; Hg, 72·7. Calc. for $C_6H_6Hg$: C, 25·9; H, 2·2; Hg, 71·9%). Its identity was confirmed (m. p. and mixed m. p.) by comparison with a sample prepared from alkaline mercuric iodide and alcoholic propyne obtained by the dehydrohalogenation of propylene bromide (Johnson and McEwen, *J. Amer. Chem. Soc.*, 1926, **48**, 472). Neither propynylmercuric chloride nor the dipropynyl compound could be reduced at ordinary temperatures. Platinum oxide catalyst used under high pressures and temperature appeared to yield propyne.

*Propylene Derivatives.*—Ethylmercuric bromide, prepared by boiling under reflux ethylmagnesium bromide (from 55 g. of ethyl bromide) with mercuric bromide for 1½ hours in an atmosphere of nitrogen, separated from ethyl alcohol in shining leaflets m. p. 193° (140 g., 90%).

Ethylmercuric bromide (50 g., 0·5 equiv.) was added in small portions to allylmagnesium bromide (Gilman and McGlumphy, *Bull. Soc. chim.*, 1928, **43**, 1322) prepared from allyl bromide (40 g.). The temperature was kept below 5° and vigorous stirring was important. When solution of the solid was complete, the product was decomposed with ice and 1% sulphuric acid, and the organic mercury compound extracted with ether and dried ($Na_2SO_4$). Removal of the solvent in a vacuum left a fawn-coloured residual liquid which was washed with absolute alcohol and kept in a vacuum-desiccator. *Allylethylmercury* (Found: Hg, 73·1, 73·5. $C_5H_{10}Hg$ requires Hg, 74·0%) decomposed when distilled, even in the presence of dry oxygen-free nitrogen, the products being diallylmercury (isolated as diallyl and mercury) and diethylmercury, b. p. 152—155° (Found: Hg, 77·8. Calc. for $C_4H_{10}Hg$: Hg, 77·9%). Allylethylmercury decomposes at room temperature also, as indicated by the formation of a grey mist of mercury. The highest yield was 60% and was obtainable only when the scale of the preparation was small. Attempts to prepare the compound from allylmercuric iodide and ethylmagnesium iodide led to the formation of ethylmercuric iodide and diethylmercury. In a second experiment allylmercuric bromide (8 g.) was added to ethereal diethylzinc (1·5 g.; *Org. Synth.*, Coll. Vol. II, p. 184). Reaction was immediate and solution took place accompanied by the formation of a mist of mercury, together with diethylmercury and diallyl.

*Reactions of Allylethylmercury.*—The liquid (1·5 g.) was dissolved in ether, and a saturated alcoholic solution of mercuric chloride was added to the boiling solution until precipitation was complete. Fractional crystallisation of the precipitate from alcohol afforded ethylmercuric chloride, m. p. 190—191° (55%), the more soluble allylmercuric chloride (45%), m. p. 110—112°, being obtained from the mother-liquors. An ethereal solution of allylethylmercury (2 g.) likewise yielded ethylmercuric chloride (70%) when warmed with alcohol (7 c.c.) saturated with hydrogen chloride. Addition of potassium iodide precipitated the remainder of the mercury as mercuric iodide.

*Boron compounds.*

Methyl borate (Etridge and Sugden, *J.*, 1928, 989) was fractionally distilled and freed from the last traces of methyl alcohol by concentrated sulphuric acid (Seaman and Johnson, *J. Amer. Chem. Soc.*, 1931, **53**, 713). *n*-Butyl borate was obtained by the method of *Org. Synth.* (Coll. Vol. II, p. 106) (Found: B, 4·6, 4·6. Calc. for $C_{12}H_{27}O_3B$: B, 4·8%). Triallyl borate (Councler,

*loc. cit.*), b. p. 77—78°/14 mm. (yield 84%) (Found : B, 6·0; 5·8. Calc. for $C_9H_{15}O_3B$ : B, 6·0%), and trimethallyl borate (Thomas, *J.*, 1946, 820, see also Ballard, B.P. 595,502/1947), b. p. 119—121°/20 mm. (yield, 87%) (Found : B, 4·9, 5·1. Calc. for $C_{12}H_{21}O_3B$ : B, 4·9%), were prepared by the latter author's method of slowly distilling a mixture of boric acid, benzene, and the appropriate alcohol until no more water came over.

*Derivatives of Diallylboric Acid.*—Boron trifluoride was prepared from potassium fluoroborate (52 g.), boron oxide (8 g.), and concentrated sulphuric acid (100 c.c.) in all-glass apparatus (Booth, *Inorg. Synth.*, **1**, 21). A slow stream of the gas was passed into a stirred ethereal solution of allylmagnesium bromide obtained from allyl bromide (80 g.), magnesium (48 g.), and ether (800 c.c.), which was cooled to −10°. After 1 hour, the product was decomposed at 0° with saturated ammonium chloride solution, and the ethereal layer was siphoned into a nitrogen-filled separating funnel. The aqueous layer was extracted with ether, and the combined extracts were dried ($Na_2SO_4$). Removal of the solvent in nitrogen afforded a faintly yellow liquid containing a small amount of inorganic material. Fractionation gave products, b. p. 90°/12 mm., 120°/12 mm., and 95°/0·2 mm., all colourless liquids with garlic odours, unsaturated to bromine water and alkaline potassium permanganate, and free from fluorine. They had nearly identical compositions (Found, respectively : C, 68·1; H, 9·9%; *M*, 356. C, 68·3; H, 10·0. C, 68·6; H, 10·9%; *M*, 403). The high molecular weights may be connected with the well-known tendency of boron oxides to form polymeric cyclic products in contact with boron trifluoride. The same substances were formed when boron trifluoride–ether complex was substituted for gaseous boron trifluoride. Repeated distillation of the two lower-boiling fractions led to dehydration and the formation of pure *bisdiallylboron oxide*, b. p. 118—120° mm. (32%) (Found : C, 71·0; H, 9·9; B, 10·6. $C_{12}H_{20}OB_2$ requires C, 71·2; H, 9·9; B, 10·9%).

*Methyl Diallylborate.*—A three-necked flask (2 l.) was fitted with a stirrer, a nitrogen-inlet concentric with its shaft, and a dropping funnel with its delivery tube bent towards the centre of the flask. The third neck carried a Y-shaped nitrogen outlet, one arm of which was connected to the dropping funnel and the other to a reflux condenser. The flask was cooled to −15° and the air displaced by the passage, for 20 minutes, of a rapid stream of oxygen-free nitrogen. A solution of methyl borate (44 g.) in ether (200 c.c.) was rapidly introduced into the flask, and previously prepared ethereal allylmagnesium bromide (from 60 g., 0·5 mol. of allyl bromide) siphoned under nitrogen into the dropping funnel. The addition of the magnesium compound caused the precipitation of a thick white addition product which, after overnight stirring in an atmosphere of nitrogen, was decomposed and extracted with ether in the usual way and dried. Distillation of the ether from a steam-bath led to partial crystallisation; water (20 c.c.) was therefore added and the distillation continued until no more volatile products were obtained. The fawn-coloured liquid residue was separated from precipitated boric acid and on distillation yielded three main fractions : (i) B. p. 82°/15 mm. (31%), *methyl diallylborate*, unchanged by repeated distillation (Found : C, 67·8, 67·9; H, 9·9, 10·2; B, 8·7. $C_7H_{13}OB$ requires C, 67·8; H, 10·4; B, 8·8%). Bisdiallylboron oxide was obtained when the ester was hydrolysed and the product distilled. (ii) B. p. 110°/0·4 mm., which, like the first fraction, was unsaturated and was probably not a methyl ester since it was also obtained from tributyl borate (below). When it was boiled with water boric acid was the sole product but the high boiling point indicated that it may have been *diallyl allylborate* (Found : C, 62·4; H, 9·6; B, 6·2. $C_9H_{15}O_2$ requires C, 65·0; H, 9·0; B, 6·6%). It may be remarked that analyses of boron compounds for carbon present great difficulty and low results are often met with in the literature; this has been attributed to the fusion of boron trioxide with the carbon particles. (iii) The third fraction, b.p. >250°/0·2 mm., was not investigated.

Substitution of triallyl borate for the methyl ester yielded a liquid, b. p. 75—76°/11 mm., which appeared to be mainly unchanged ester. Allyl alcohol was also formed. Use of tri-*n*-butyl borate led to a small amount of a colourless viscous oil, b. p. 111°/0·1 mm., insoluble in water but soluble in alkali. Analysis indicated its *possible* identity with (ii) described above (Found : C, 62·0; H, 8·7; B, 5·0%). The quantity was too small for extensive attempts to determine its structure and no method for obtaining it in larger amounts was successful.

*Reaction of Allylmagnesium Bromide with Di-n-butylboron Bromide.*—In the apparatus previously described, allylmagnesium bromide (1 equiv.) was treated with di-*n*-butylboron bromide (14 g.; Johnson, Snyder, and Van Campen, *loc. cit.*). The products were tri-*n*-butylborine, b. p. 86°/20 mm. (8 g.) (Found : B, 5·9. Calc. for $C_{12}H_{27}B$ : B, 6·0%), and bisdiallylboron oxide (2 g.) (Found : C, 70·9; H, 9·7. Calc. for $C_{12}H_{20}OB_2$ : C, 71·2; H, 9·9%).

*Reactions with Diethylzinc.*—The reagent, purified by distillation in carbon dioxide, had b. p. 117—119°.

(*a*) ***In tetralin.*** A solution of triallyl borate (20 g., 1 equiv.) in purified tetralin (20 g.) was slowly added to a vigorously stirred mixture of diethylzinc (41 g., 3 equivs.) and tetralin (40 g.), carbon dioxide being passed through the liquid. Little reaction occurred at 0° and the flask was heated at 100° for 2 hours. Fractionation of the product afforded fractions, (i) b. p. 85—95° (2 g.), and (ii) b. p. 130—140° (8 g.). Both these liquids had a pronounced phenolic odour, contained combined boron, and were unsaturated. The lower-boiling reacted vigorously with sodium but no indication of free allyl alcohol was obtained. These substances were insoluble in water but dissolved in sodium hydroxide, the second fraction with evolution of heat. Fraction (i) gave analytical figures corresponding to the diethyl ethylborate (b. p. 125°) though its b. p. was nearer to that of ethyl diethylborate (Found: C, 55·3; H, 11·5; B, 7·8. Calc. for $C_6H_{15}O_2B$: C, 55·4; H, 11·5; B, 8·5%). However, its stability to water was greater than that of either of these two esters. Fraction (ii) was probably, in spite of the low analysis for carbon, *allyl diethylborate* (Found: C, 64·0; H, 11·8; B, 8·3. $C_7H_{15}OB$ requires C, 66·6; H, 11·9; B, 8·7%). Hydrolysis with sodium hydroxide followed by acidification yielded a saturated acid which on distillation furnished bisdiethylboron oxide, b. p. 143° (Meerwein and Sönke, *J. pr. Chem.*, 1936, **147**, 251).

(*b*) The experiment was repeated in the absence of a solvent (Councler, *loc. cit.*). A colourless liquid, b. p. 115—120°, was isolated (Found: B, 7·8. $C_9H_{15}OB$ requires B, 7·9%). The figure for boron, in conjunction with Councler's analytical figures for carbon and hydrogen, indicate the substance to be *ethyl diallylborate*.

(*c*) Experiment (*a*) was repeated with trimethallyl borate (32 g.) in tetralin (30 g.), and diethylzinc (53 g.) in the same solvent (20 g.). A large part of the methallyl alcohol (20 g.) was recovered unchanged, together with a liquid, b. p. 52—54°/17 mm. (3 g.), which appeared to be *methallyl diethylborate*, though the analytical value for hydrogen was not altogether satisfactory (Found: C, 68·9; H, 13·1; B, 7·5. $C_8H_{17}OB$ requires C, 68·5; H, 12·1; B, 7·8%).

*Di*-n-*butyl* 3-*methoxypropylborate* was prepared by addition of tri-*n*-butyl borate to one equivalent of the Grignard reagent prepared from 3-methoxypropyl chloride (Noyes, *Amer. Chem. J.*, 1897, **19**, 767; Palomaa and Jansen, *Ber.*, 1931, **64**, 797; 1938, **71**, 597). It had b. p. 140—150°/41 mm., the yield being 20% (Found: C, 62·2; H, 12·1; B, 4·9. $C_{10}H_{21}O_3B$ requires C, 62·6; H, 11·7; B, 4·8%).

Nearly all the analyses, except for those for mercury and boron, were carried out by Drs. Weiler and Strauss, Oxford. The Department of Scientific and Industrial Research is thanked for a grant in aid of this research.

THE UNIVERSITY, LEEDS. [*Received, April 8th*, 1952.]

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## 572. *Eight- and Higher-membered Ring Compounds. Part IX.* Improved Synthesis of Coronene.*

By WILSON BAKER, J. F. W. MCOMIE, and W. K. WARBURTON.

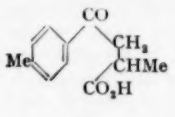
The synthesis of coronene described in Part V (*J.*, 1951, 1118) from 2 : 7-dimethylnaphthalene (III) *via* di(naphthalene-2 : 7-dimethylene) (IV) has been improved.

IN Part V of this series (Baker, Glockling, and McOmie, *J.*, 1951, 1118) was described a synthesis of coronene from 2 : 7-dimethylnaphthalene (III), *via* di(naphthalene-2 : 7-dimethylene) (IV). Improvements in this synthesis are now reported.

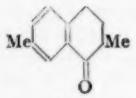
2 : 7-Dimethylnaphthalene (III) may be prepared in five steps from toluene and methylsuccinic anhydride in 50% (reproducible) yield. The most critical stage, the formation of di(naphthalene-2 : 7-dimethylene) (IV) from 2 : 7-bisbromomethylnaphthalene, was previously brought about in 16·3% yield by reaction with sodium in dioxan, but the isolation of (IV) in the pure state was tedious owing to the necessity of hand-picking it from 1 : 2-di-(7-methyl-2-naphthyl)ethane (V) which was simultaneoulsy formed in some 4% yield. By working in more dilute solution (100 c.c. of dioxan for each 1 g. of the dibromocompound, instead of the approximately 20 c.c. of dioxan previously employed) the yield

* Part VIII, *J.*, 1952, 1452.

of (IV) unexpectedly fell to 4·4%, the major product being (V). A considerable improvement has now been effected in the Würtz–Fittig type reaction by adding a benzene solution of the dibromide to a solution of phenyl-lithium in ether; a 20% yield of (IV) was readily isolated, and none of the compound (V) was produced. The final yield of coronene is 5% from 2 : 7-dimethylnaphthalene in three steps, or 2·5% in eight steps from toluene. The synthesis of coronene due to Newman (*J. Amer. Chem. Soc.*, 1940, **62**, 1683) gave a 1·7% yield in six steps from 7-methyltetralone.



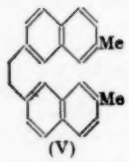

(I)




(II)

(III)

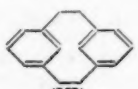
(IV)




(V)

Methylsuccinic acid was prepared from methyl methacrylate by reaction with sodium cyanide and hydrolysis, and the derived anhydride was condensed with toluene to give β-*p*-toluoyl*iso*butyric acid (I), which was then reduced by Clemmensen's method to α-methyl-γ-*p*-tolylbutyric acid. Cyclisation of α-methyl-γ-*p*-tolylbutyric acid with technical fluorosulphonic acid in carbon disulphide gave 2 : 7-dimethyltetralone (II) in very high yield, and a further Clemmensen reduction followed by catalytic dehydrogenation gave 2 : 7-dimethylnaphthalene (III). The bromination of (III) with *N*-bromosuccinimide, and the final conversion of (IV) into coronene, were carried out as previously described.

Although only indirectly concerned with the present synthesis of coronene, the opportunity may be taken to add some observations relating to the similar synthesis of pyrene from *m*-xylylene dibromide *via* di-*m*-xylylene (VI) (Part IV of this series, Baker, McOmie, and Norman, *J.*, 1951, 1114). The possibility existed that di-*m*-xylylene (rhombs, m. p. 132—133°), which is easily dehydrogenated to pyrene and isomerised by aluminium chloride to *as*-1 : 2 : 2*a* : 3 : 4 : 5-hexahydropyrene, might be identical with *s*-3 : 4 : 5 : 8 : 9 : 10-hexahydropyrene (needles, m. p. 131—133°; Cook and Hewett, *J.*, 1933, 404), but this is excluded by the fact that a mixture of the two compounds melted over the range 90—97°. Moreover *s*-hexahydropyrene gives a red-brown picrate, whilst



(VI)

(VII)

(VIII)

di-*m*-xylylene forms no picrate. We are greatly indebted to Professor J. W. Cook, F.R.S., for specimens of *s*- and *as*-hexahydropyrene.

## Experimental

*Methylsuccinic Acid and Anhydride.*—This is conveniently prepared (61% yield) from commercial methyl methacrylate by reaction with sodium cyanide and subsequent hydrolysis, following the directions given in *Org. Synth.* (**26**, 54) for its preparation from ethyl crotonate. The final extraction of the methylsuccinic acid is best carried out by ethyl acetate instead of ether. The anhydride (b. p. 127—129°/30 mm.; 83% yield) was prepared from the acid by reaction with acetyl chloride (Dev and Guha, *J. Indian Chem. Soc.*, 1948, **25**, 16).

α-*Methyl-γ*-p-*tolylbutyric Acid.*—β-*p*-Toluoyl*iso*butyric acid (I) was prepared in 80—83% yield from methylsuccinic anhydride, toluene, and aluminium chloride in nitrobenzene (Dev, *J. Indian Chem. Soc.*, 1948, **25**, 69). Reduction of the keto-acid for 36 hours by the

Clemmensen–Martin method with the addition of acetic acid (5 c.c. for each 50 g. of the keto-acid) gave α-methyl-γ-*p*-tolylbutyric acid, m. p. 54—56°, b. p. 143—144·5°/0·8 mm., in 95% yield (Bailey, Bryant, Hancock, Morrell, and Smith, *J. Inst. Petroleum*, 1947, **33**, 503, record m. p. 50—51°, b. p. 150°/0·8 mm.).

2 : 7-*Dimethyltetralone* (II).—To α-methyl-γ-*p*-tolylbutyric acid (15 g.) in carbon disulphide (50 c.c.) was added during 15 minutes with vigorous stirring technical fluorosulphonic acid (13 c.c.; containing some free sulphur trioxide). The mixture was then boiled for 1 hour, cooled, and poured into ice and water, and excess of sodium carbonate added. Extraction with ether then yielded 2 : 7-dimethyltetralone (II) (13·1 g., 97%), b. p. 93—95°/0·3 mm., giving a 2 : 4-dinitrophenylhydrazone, m. p. 251° (Bailey *et al.*, *loc. cit.*, record b. p. 122°/0·5 mm.; 2 : 4-dinitrophenylhydrazone, m. p. 251°).

2 : 7-*Dimethyltetralin*.—Clemmensen–Martin reduction of the tetralone as in the case of the keto-acid (above) gave 2 : 7-dimethyltetralin, b. p. 80—84°/1 mm. (85%) (Bailey *et al.*, *loc. cit.*, record b. p. 95—110°/0·27 mm.).

2 : 7-*Dimethylnaphthalene* (III).—The dimethyltetralin was dehydrogenated in the vapour phase at 30 mm. over a charcoal catalyst containing 0·5% each of palladium and platinum. The charcoal column (1 × 35 cm.) was kept at 440°, and the dimethyltetralin introduced at the rate of 5 c.c. per hour. 2 : 7-Dimethylnaphthalene was obtained directly (86% yield), of m. p. and mixed m. p. with an authentic specimen, 95·5—96° (picrate, m. p. 135·5—136°).

*Di*(*naphthalene*-2 : 7-*dimethylene*) (IV).—To a stirred solution of phenyl-lithium prepared from lithium (0·70 g.) and bromobenzene (7·8 g.) in anhydrous ether (100 c.c.) was added in an atmosphere of nitrogen during 2 hours a solution of 2 : 7-bisbromomethylnaphthalene (Part V, Baker, Glockling, and McOmie, *loc. cit.*) (6·24 g.) in benzene (700 c.c.). The temperature was then raised to 60° and stirring continued for ½ hour; the mixture was cooled, water added, and the whole shaken and filtered, leaving some benzene-insoluble material (*ca.* 1 g.). The washed benzene solution was then distilled to dryness under reduced pressure, and finally heated at 150°/20 mm. for 2 hours to remove most of the bromobenzene. The solid residue was sublimed on to a cold finger, at temperatures rising to 250°/0·1 mm., and was then resublimed. After ½ hour at 120°/0·5 mm. was obtained low-melting material (0·2 g.) containing a trace of bromobenzene, followed at 220°/0·5 mm. by a solid (m. p. >180°), which after one crystallisation from benzene gave di(naphthalene-2 : 7-dimethylene) (IV) as either long, thick needles, or hexagonal plates, m. p. 235—236·5° (628 mg., 20%), identical (mixed m. p.) with the substance previously described, m. p. 237—238°.

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THE UNIVERSITY, BRISTOL. [*Received, May 3rd*, 1952.]

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## 573. *The Thermodynamics of Dissociating Solvents.*

By K. W. DUNNING and W. J. DUNNING.

The thermodynamic properties of solvents which dissociate are examined. It is shown that if a certain species is present in a conventionally pure solvent as a result of dissociation, then no matter how complicated the equilibria may be which involve that species, $(\partial\mu_s/\partial m)_0$ of the solvent is zero. Other consequences of the dissociation are discussed.

IT is well known that if the two components of a binary system form a chemical compound in both the liquid and the solid state, then the melting point–composition curve shows a maximum at the stoicheiometric point of the compound and that this maximum is more or less rounded, the greater or smaller the degree of dissociation of the compound. Theoretical consideration (H. A. Lorentz in Stortenbeker, *Z. physikal. Chem.*, 1892, **10**, 183; Lewis, *ibid.*, 1908, **61**, 129; Van Laar, *ibid.*, 1909, **66**, 197) has been confined to the discussion of one simple equilibrium between the two components. Recent interest in this topic (Dunning and Nutt, *Trans. Faraday Soc.*, 1951, **47**, 15; Gillespie, *J.*, 1950, 2493; Gillespie, Hughes, and Ingold, *J.*, 1950, 2552) presents the converse problem. A solvent is suspected

of dissociation. The dissociation products are not in the first instance known and they may be interrelated by any number of equilibria. Without a detailed knowledge of the products and the equilibria involved, the former methods of proof are inapplicable. A proof is offered which depends only on the fact that if a certain species is present in the conventionally pure solvent, then $(\partial\mu/\partial m)_0$ of that species is not infinite. This appears in the second part of the paper. In the first part, at the suggestion of Professor E. A. Guggenheim, the approaches of Lewis and of Van Laar are extended to more complicated systems and not only is the proof more satisfactory, but it can be readily extended to any dissociating solvent, provided the products and equilibria are known. It is limited to slightly dissociated solvents.

1. In the first procedure, it is convenient to consider, as an example, the following equilibria in nitric acid:

$$2HNO_3 \rightleftharpoons NO_2^+ + H_2O + NO_3^- \quad . . . . . (1)$$

$$H_2O + HNO_3 \rightleftharpoons H_3O^+ + NO_3^- \quad . . . . . (2)$$

Quantities referring to the species $NO_2^+$, $H_2O$, $NO_3^-$, and $H_3O^+$ will be designated by the subscripts $n^+$, h, $n^-$, and $h^+$. In the same way, $n_s$ is the number of moles of the species $HNO_3$ which are present and is distinct from $n_o$, the number of moles of conventional nitric acid originally present. Suppose now that $n_w$ moles of water are added to the $n_o$ moles of nitric acid, the following relations apply during the additions;

$$n_s + 2n_h + 3n_{h^+} = n_o + 2n_w \text{ (Hydrogen-atom balance)} \quad . . . (3)$$

$$n_s + n_{n^+} + n_{n^-} = n_o \text{ (Nitrogen-atom balance)} \quad . . . . . (4)$$

$$n_{n^+} - n_{n^-} + n_{h^+} = 0 \text{ (Charge balance)} \quad . . . . . (5)$$

$$2d\mu_s = d\mu_{n^+} + d\mu_h + d\mu_{n^-} \text{ (Equilibrium 1)} \quad . . . (6)$$

$$d\mu_h + d\mu_s = d\mu_{h^+} + d\mu_{n^-} \text{ (Equilibrium 2)} \quad . . . . . (7)$$

$$n_s d\mu_s + n_{n^+} d\mu_{n^+} + n_{n^-} d\mu_{n^-} + n_h d\mu_h + n_{h^+} d\mu_{h^+} = 0 \text{ (Gibbs–Duhem)} \quad . (8)$$

By putting $m_{n^+} = n_{n^+}/n_s$ and $d\mu_{n^+} = \log m_{n^+}$, etc., we obtain from (8),

$$d\mu_s + \boldsymbol{R}T(dm_{n^+} + dm_h + dm_{n^-} + dm_{h^+}) = 0 \quad . . . . . (9)$$

from (6),

$$d\mu_s - \frac{\boldsymbol{R}T}{2}\left(\frac{dm_{n^+}}{m_{n^+}} + \frac{dm_h}{m_h} + \frac{dm_{n^-}}{m_{n^-}}\right) = 0 \quad . . . . . (10)$$

and from (7),

$$d\mu_s - \boldsymbol{R}T\left(\frac{-dm_h}{m_h} + \frac{dm_{n^-}}{m_{n^-}} + \frac{dm_{h^+}}{m_{h^+}}\right) = 0 \quad . . . . . (11)$$

Equations (3) and (4) give

$$-dm_{n^+} + 2dm_h - dm_{n^-} + 3dm_{h^+} - 2dm_w = 0 \quad . . . . . (12)$$

and (5) gives

$$dm_{n^+} - dm_{n^-} + dm_{h^+} = 0 \quad . . . . . (13)$$

The solution of these is

$$d\mu_s/dm_w = -2\boldsymbol{R}T m_{n^-} m_w/(m_{n^+}m_{n^-} + 4m_{h^+}m_{n^+} + m_{n^-}m_{h^+} + m_{n^+}m_h + m_{n^-}m_h + m_h m_{h^+} + 12m_{n^+}m_{n^-}m_{h^+} + 2m_{n^+}m_h m_{h^+} + 6m_{n^+}m_{n^-}m_h + m_{n^-}m_h m_{h^+}) \quad (14)$$

As $m_w$ approaches zero, $m_{n^-}$ etc. all approach their values in the pure dissociated acid. Since these values are all finite and positive at $m_w = 0$, it is clear that

$$d\mu_s/dm_w \longrightarrow 0 \text{ as } m_w \longrightarrow 0 \quad . . . . . (15)$$

By substituting for $m_{n^+}$ and $m_{n^-}$, it is easily shown that equation (14) reduces to

$$d\mu_s/dm_w = -2\boldsymbol{R}T \quad . . . . . (16)$$

when excess of water is present. Under these conditions, the change in chemical potential of the solvent with change in the concentration of added solute is that of a solute which dissociates into two ions.

The relationships (15) and (16) may be used to obtain the dependence of the osmotic properties of these solvents and solutes on the amount of solute added. Hence, it is found from (15) that, if a solvent dissociates, then the addition of one of the dissociation products

(water here) does not initially alter the freezing point. From (16) it is clear that at sufficiently high concentrations of this solute, the freezing point curve follows that predicted from van't Hoff's argument for $i = 2$.

2. The same conclusions follow from another argument. In this no explicit calculations are made in which a knowledge of the equilibria present is necessary; the argument is therefore more general, and only the fact that equilibria are present is used.

We shall assume that a solvent (nitric acid) dissociates into certain species ($H_2O$, $NO_2^+$, etc.). If to $n_o$ moles of conventional nitric acid a small amount $n_w$ moles of water is added, then since the Gibbs free energy $G$ of the system is a homogeneous function of the first degree in $n_o$ and $n_w$, we obtain

$$n_o d\mu_s + n_w d\mu_h = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (17)$$

Equation (17) can also be obtained from (8) by using (3), (4), and (5) to eliminate $n_{n^+}$, $n_{n^-}$, and $n_h$. There results the equation

$$n_s(2d\mu_s - d\mu_{n^+} - d\mu_h - d\mu_{n^-}) + n_{h^+}(-d\mu_{n^+} - 3d\mu_h + d\mu_{n^-} + 2d\mu_{h^+}) + n_o(d\mu_{n^+} + d\mu_h + d\mu_{n^+}) + 2n_w d\mu_h = 0$$

and comparison of the parenthetical terms with (6) and (7) then gives (17).

Equation (17) is the Gibbs–Duhem equation in a form which would be used were one unaware of the dissociative process, only the suffixes indicate that a dissociation is under consideration. Now

$$\mu_s = \mu_{s_o} + \int_0^{n_w} \frac{\partial \mu_s}{\partial n_w} \cdot dn_w \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (18)$$

and by using (17)

$$\mu_s = \mu_{s_o} - \int_0^{n_w} \frac{n_w}{n_o} \frac{\partial \mu_h}{\partial n_w} \cdot dn_w \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (19)$$

By putting

$$\frac{\partial \mu_h}{\partial n_w} = \left(\frac{\partial \mu_h}{\partial n_w}\right)_0 + \left(\frac{\partial^2 \mu_h}{\partial n_w^2}\right)_0 n_w \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (20)$$

where the subscript 0 designates the values in pure nitric acid when $n_w = 0$, we now have

$$\mu_s = \mu_{s_o} - \int_0^{n_w} \frac{n_w}{n_o} \left\{\left(\frac{\partial \mu_h}{\partial n_w}\right)_0 + \left(\frac{\partial^2 \mu_h}{\partial n_w^2}\right)_0 n_w\right\} dn_w \quad . \quad . \quad . \quad . \quad (21)$$

$$= \mu_{s_o} - \left(\frac{\partial \mu_h}{\partial n_w}\right)_0 \frac{n_w^2}{2n_o} - \left(\frac{\partial^2 \mu_h}{\partial n_w^2}\right)_0 \frac{n_w^3}{3n_o} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

The Taylor expansion (20) is permissible only when the solvent dissociates and the expanded quantity ($\partial \mu_h / \partial n_w$ in this case) refers to one of the dissociation products; only in this case are the derivatives at $n_w = 0$ finite.

If we put

$$\mu_h = {}_o\mu_h + RT \log (f_h n_h / \Sigma n) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$

with $f_h$ an activity coefficient and ${}_o\mu_h$ the chemical potential of water in a solution of unit activity, then

$$\left(\frac{\partial \mu_h}{\partial n_w}\right)_0 = RT\left\{\left(\frac{1}{n_h}\right)_0 - \left(\frac{1}{\Sigma n}\right)_0\right\}\left(\frac{\partial n_h}{\partial n_w}\right)_0 + RT\left(\frac{\partial \log f_h}{\partial n_w}\right)_0 \quad . \quad . \quad (24)$$

It is necessary to comment that an alternative expression to (23) might have been chosen; for example,

$$\mu_h = {}_o\mu_w + RT \log [f_w n_w / (n_o + n_w)] \quad . \quad . \quad . \quad . \quad . \quad (25)$$

However, since the chemical potential of water is finite in pure nitric acid, this means that $f_w$ must become infinite when $n_w$, the quantity of added water, becomes zero. This is inconvenient, and it is therefore better to choose (23) in which $f_h$ will approach unity as $n_h$ becomes small; of course $f_h$ will probably still not be unity when $n_w = 0$, unless ${}_o\mu_h$ is chosen suitably. If ${}_o\mu_h$ were so chosen, $f_h$ might then be different from unity in solutions where $n_h < (n_h)_o$, for example, in solutions where the dissociation has been suppressed by addition of dinitrogen pentoxide, but this is not a serious disadvantage. In other words, it is not possible to use an infinitely dilute solution of water in nitric acid as the norm, as is

customary.* We will therefore use (23) as the expression for $\mu_h$ and choose ${}_0\mu_h$ so that $f_h = 1$ when $n_w = 0$. With this choice it is to be expected that $(\partial \log f_h/\partial n_w)_0$ will be finite, but in most cases it will be small. Equation (24) can then be replaced by the approximation

$$\left(\frac{\partial \mu_h}{\partial n_w}\right)_0 \doteq RT\left(\frac{1}{n_h}\cdot\frac{\partial n_h}{\partial n_w}\right)_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (26)$$

By referring now to (22), it is seen that $(\partial\mu_s/\partial n_w)_0$ will be zero. The addition of small traces of water to pure nitric acid will not alter its chemical potential, whilst $(\partial^2\mu_s/\partial n_w^2)_0$, the curvature, will be given by equation (26); that is

$$(\partial\mu_s/\partial n_w)_0 = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (27)$$

and

$$\left(\frac{\partial^2\mu_s}{\partial n_w^2}\right)_0 \doteq \frac{RT}{n_0}\left(\frac{1}{n_h}\cdot\frac{\partial n_h}{\partial n_w}\right)_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (28)$$

To assess the significance of (28) the influence of the terms on the right may be examined; if the degree of dissociation is small $(1/n_h)_0$ will be large and $(\partial n_h/\partial n_w)_0$ will be just less than unity. Hence the curvature of the $\mu_s$–$n_w$ function at $n_w = 0$ will be large. On the other hand, if the degree of dissociation is large $(1/n_h)_0$ will be smaller and $(\partial n_h/\partial n_w)_0$ will be much less than unity, and so the curvature will be small.

If now, instead of water, $n_p$ moles of dinitrogen pentoxide had been added to the pure nitric acid, instead of (17) there would be obtained

$$n_0 d\mu_s + n_p(d\mu_{n^+} + d\mu_{n^-}) = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (29)$$

on the assumption that the pentoxide dissociates into $NO_2^+$ and $NO_3^-$ ions. Whence,

$$\mu_s = \mu_{s_0} - \left(\frac{\partial[\mu_{n^+} + \mu_{n^-}]}{\partial n_p}\right)_0 \cdot \frac{n_p^2}{2n_0} - \frac{\partial^2(\mu_{n^+} + \mu_{n^-})}{\partial n_p^2}\cdot\frac{n_p^3}{3n_0} \quad . \quad . \quad . \quad . \quad (30)$$

If complete ionization of the dinitrogen pentoxide is assumed, the curvature is given by

$$\left(\frac{\partial[\mu_{n^+} + \mu_{n^-}]}{\partial n_p}\right)_0 = RT\left\{\frac{n_{n^+} + n_{n^-}}{n_{n^+}n_{n^-}} - \frac{2}{\Sigma n}\right\}\left(\frac{\partial[n_{n^+} + n_{n^-}]}{\partial n_p}\right)_0 + RT\left(\frac{\partial \log f_{n^+}f_{n^-}}{\partial n_p}\right)_0 \quad (31)$$

If only the simple dissociation (1) is considered, $(n_{n^+})_0 = (n_{n^-})_0 = (n_h)_0$; comparison of (31) with (24) then shows that the curvature of the $\mu_s$–$n_p$ function at $n_p = 0$ will be about twice that of the $\mu_s$–$n_w$ curve at $n_w = 0$. The additional equilibrium (2) complicates the issue, but there is another clue to the dissociation of the pentoxide. When the amount of added water or dinitrogen pentoxide greatly exceeds the amounts of the species present in the pure acid, that is, when

$$n_w \gg (n_h)_0 \text{ or } n_p \gg (n_{n^+})_0 \text{ and } (n_{n^-})_0,$$

then

$$n_w \simeq n_h \text{ or } n_p \simeq n_{n^+} \text{ and } n_{n^-}$$

and equations (17) and (29) become

$$n_0\partial\mu_s/\partial n_h + n_h\partial\mu_h/\partial n_h = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (32)$$

and

$$2n_0\partial\mu_s/\partial n_{n^-} + n_{n^-}\partial(\mu_{n^+} + \mu_{n^-})/\partial n_{n^-} = 0 \quad . \quad . \quad . \quad . \quad (33)$$

These are the equations of the asymptotes which the $\mu_s$–$n_w$ or $\mu_s$–$n_p$ curves approach as $n_s$ or $n_w$ increases, and they are the conventional relations for non-dissociating and binary dissociating solutes, respectively. By examining the limiting behaviour for relatively large additions of dinitrogen pentoxide, a decision can be made regarding dissociation. Should there be additional equilibria such as

$$2HNO_3 \rightleftharpoons N_2O_5 + H_2O \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (34)$$

and

$$N_2O_5 \rightleftharpoons NO_2^+ + NO_3^- \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (35)$$

the disentangling becomes more difficult. The experimental data would have to be accurate to the third order of differentiability, and deviations due to the non-ideality would become significant.

* The best procedure for detailed calculations would be to refer the chemical potentials of water in the solutions and in the pure acid to the $\mu_w^0$ of the water vapour. This procedure would, however, complicate the present discussion.

Considering now the addition of an inert solute C to the pure nitric acid, it will be clear by analogy with the preceding discussion that a normal behaviour is to be expected, since for an inert solute $\partial\mu_c/\partial n_c$ becomes infinite as $n_c$ approaches zero. Hence in such solutions $\mu_s$ will decrease linearly with $n_c$ in the manner usual for dilute solutions.

Let us consider finally the addition of a solute such as potassium nitrate, which dissociates into one ion, $K^+$, which is inert, and another, $NO_3^-$, which takes part in the equilibrium. For this case

$$n_o\,\partial\mu_s/\partial n_k + n_k\,\partial(\mu_k + \mu_{n^-})/\partial n_k = 0 \quad . \quad . \quad . \quad . \quad . \quad (36)$$

and it can be shown that

$$\mu_s = \mu_{s_o} - \frac{RT}{n_o}\left(n_k - \frac{n_k^2}{2\Sigma n} + \int_0^{n_k} \frac{\partial \log f_k}{\partial \log n_k}\,.\,dn_k\right) - \left(\frac{\partial\mu_{n^-}}{\partial n_k}\right)_0 \frac{n_k}{2n_o} - \left(\frac{\partial^2\mu_{n^-}}{\partial n_k^2}\right)_0 \frac{n_k^3}{3n_o} \quad (37)$$

Expression (37) indicates that the limiting slope of the $\mu_s$–$n_k$ curve for a solute such as potassium nitrate is finite, and the tangent at $n_k = 0$ has a slope equal to that of a normal solute. In other words, at the origin the $NO_3^-$ ion is ineffective and only the $K^+$ ion has influence. Hence, over a limited range of concentration, potassium nitrate would *appear* to be undissociated and only at higher concentrations would it behave as a dissociated electrolyte.

These results show that, however complicated the dissociation of a solvent may be, the addition of one of the dissociation products does not initially alter the chemical potential of the solvent. The curvature at the origin gives some indication of the amount of that species already present as a result of the dissociation. This curvature is enhanced if the added material itself dissociates on addition and all its products take part in the dissociation of the solvent. When the added material dissociates into $x$ and $y$ particles, of which $x$ take part in the dissociation of the solvent and $y$ do not, then the initial slope of the $\mu_s$–concentration curve is $-yRT$.

For higher concentrations of added material, the dissociation of the solvent is suppressed and the rate of change in chemical potential with concentration approaches normal behaviour.

Our thanks are due to Professor E. A. Guggenheim, F.R.S., for helpful discussion.

UNIVERSITY OF BRISTOL. [*Received, March 10th*, 1952.]

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## 574. *Amides of Vegetable Origin. Part I. Stereoisomeric* N-iso*Butylundeca*-1 : 7-*diene*-1-*carboxyamides and the Structure of Herculin.*

By L. CROMBIE.

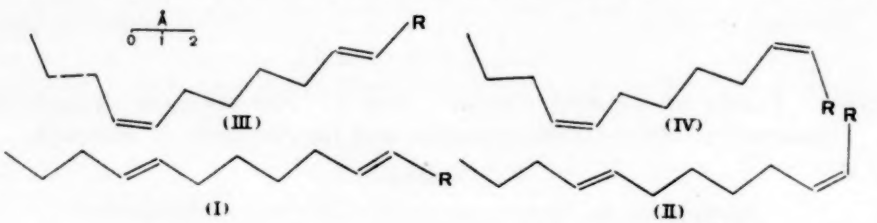
Syntheses of the *trans*-1 : *trans*-7- and *cis*-1 : *trans*-7-stereoisomers of *N-iso*butylundeca-1 : 7-diene-1-carboxyamides are recorded. The assigned configurations of these and the two other possible geometrical isomers, previously described, are supported by consideration of their infra-red spectra. None of them is identical with herculin which has therefore been assigned a wrong gross structure (stereochemistry undefined). Attempted re-isolation of herculin from the bark of *Xanthoxylum clava-herculis* L. failed, though from the cognate fraction a monosubstituted amide was obtained with certain properties similar to those recorded for the former. The new compound is more unsaturated than herculin as its ultra-violet spectrum reveals conjugated triethenoid unsaturation, and it is named *neo*herculin. The question of the nature of herculin remains open.

SEVERAL aliphatic *iso*butylamides have been isolated from vegetable sources by various authors. Amongst the better known are pellitorine obtained from the roots of *Anacyclus pyrethrum* DC. (Gulland and Hopton, *J.*, 1930, 6; Jacobson, *J. Amer. Chem. Soc.*, 1949, **71**, 366), herculin from the bark of *Xanthoxylum clava-herculis* L. (Jacobson, *ibid.*, 1948, **70**, 4234), affinin from the roots of *Heliopsis longipes* A. Gray (Acree, Jacobson, and Haller,

*J. Org. Chem.*, 1945, **10**, 236, 449), and scabrin from the roots of *Heliopsis scabra* Dunal. (Jacobson, *J. Amer. Chem. Soc.*, 1951, **73**, 100). Spilanthol has been isolated from the flowers of *Spilanthes oleracea* Jacquin and *Spilanthes acmella* (Asano and Kanematsu, *Ber.*, 1932, **65**, 1602; Gokhale and Bide, *J. Indian Chem. Soc.*, 1945, **22**, 250), whilst Japanese investigators have separated two aliphatic *iso*butylamides, shanshoöls I and II, from the fruits and bark of *Xanthoxylum piperitum* DC. (Murayama and Shinozaki, *J. Pharm. Soc. Japan*, 1931, **51**, 379; Aihiara, *ibid.*, 1950, **70**, 43, 47). There is scattered evidence that, as judged from the properties of the crude plant extracts, a number of similar compounds is to be found in other species of these genera. All the compounds mentioned have a characteristic pungent taste : when placed on the tongue a tingling sensation is caused accompanied by profuse salivation. Interest in them has quickened since several have been shown to possess considerable insecticidal activity, sometimes surpassing that of the pyrethrins (against houseflies). Structural investigation within the group is incomplete but all those known are *iso*butylamides of acids containing an unbranched sequence of ten, twelve, or eighteen carbon atoms with two or more double bonds. They are unstable in air at room temperature, though less so in solution.

None of the structures proposed for these compounds has been confirmed by synthesis, and their stereochemistry is unknown in all cases. The investigation described in this paper was aimed at verifying the proposed gross structure for herculin * (Jacobson, *loc. cit.*), *N-iso*butylundeca-1 : 7-diene-1-carboxyamide, and elucidating its stereochemistry by preparation of the four possible isomers, *viz.* : *trans*-1 : *trans*-7 (I*a*), *cis*-1 : *trans*-7 (II*a*), *trans*-1 : *cis*-7 (III*a*), and *cis*-1 : *cis*-7 (IV*a*). During this work preparation of the *trans*-1 : *cis*-7- and the *cis*-1 : *cis*-7-compound was reported (Raphael and Sondheimer, *J.*, 1950, 115; 1951, 2693), so attention was concentrated on synthesis of the remaining pair and on examination of the four compounds to confirm their stereochemistry and relate them to natural herculin.

As the key synthetical intermediate *trans-n*-non-5-en-1-ol was required. Oct-4-en-1-ol can be prepared in excellent yield by the ring scission of the readily available 3-chlorotetrahydro-2-*n*-propylpyran with metallic sodium and is known to have the *trans*-configuration (Riobé, *Ann. Chim.*, 1949, **4**, 593; Crombie and Harper, *J.*, 1950, 1707).



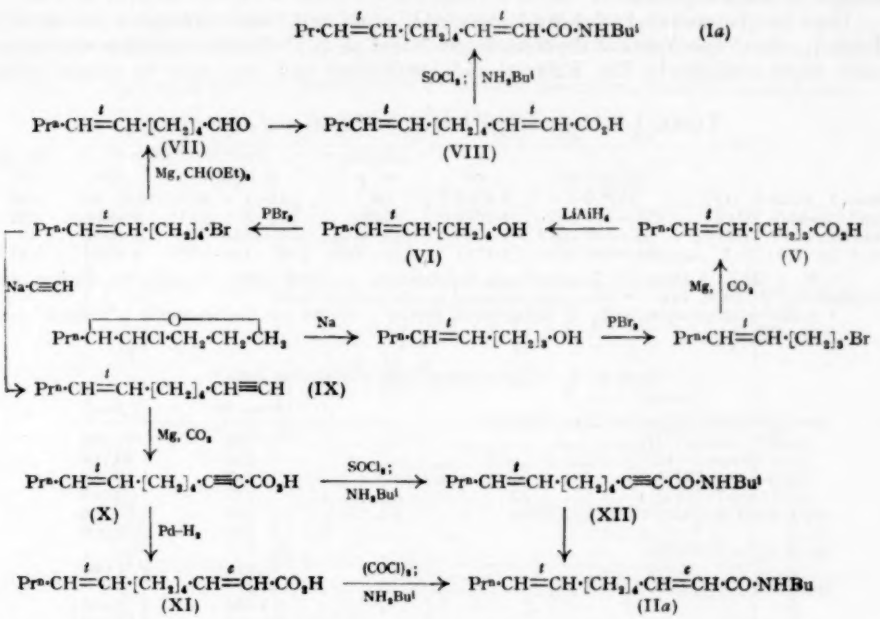

(*a*) R = ·CO·NH·$CH_2$·$CHMe_2$ (*b*) R = ·$CO_2^-$ $C(NH_2)(NH_2^+)$S·$CH_2$Ph

By a standard homologation procedure this alcohol was converted *via* its bromide and the derived Grignard reagent into *trans-n*-oct-4-ene-1-carboxylic acid (V) (*S*-benzylthiuronium salt) which was reduced with lithium aluminium hydride to *trans-n*-non-5-en-1-ol (VI) (*p*-diphenylylurethane), the overall yield being 47%.

For the preparation of undeca-*trans*-1 : *trans*-7-diene-1-carboxylic acid, the nonenol was converted into its bromide with phosphorus tribromide, and the derived Grignard reagent treated with ethyl orthoformate. Hydrolysis of the resultant acetal yielded *trans-n*-non-5-en-1-al (VII) (2 : 4-dinitrophenylhydrazone), purified by means of its bisulphite compound. This aldehyde condensed with malonic acid in pyridine at room temperature during five days, to give the required diene acid (VIII) (*S*-benzylthiuronium salt) which

* To avoid confusion it is pointed out that the source of herculin, *Xanthoxylum clava-herculis* L., is also referred to in the literature as *X. carolinianum* Lam., *Fagara caroliniana* Engler, southern prickly ash, Hercules club, and toothache tree.

crystallised in colourless plates when distilled. Evidence that a double bond introduced into an acid by means of the Doebner reaction has the *trans*-configuration has been cited by numerous authors (von Auwers and Wissenbach, *Ber.*, 1923, **56**, 715; Letch and Linstead,




*J.*, 1932, 443; Schjanberg, *Ber.*, 1937, **70**, 2385; Lauer and Gensler, *J. Amer. Chem. Soc.*, 1945, **57**, 1171). On the other hand Joly and Amiard (*Bull. Soc. chim.*, 1947, 139) state that, dependent on conditions, 6—17% of (*cis*-)hexenolactone can be isolated in the preparation of sorbic acid by this reaction, though this of course does not *necessitate* a *cis*-αβ-precursor. In the present instance the crystallinity of the purified acid indicates the absence of *cis*-contaminant. When treated with thionyl chloride and an excess of *iso*butylamine, *N-iso*butylundeca-*trans*-1 : *trans*-7-diene-1-carboxyamide (I*a*) was formed which when distilled crystallised in waxy needles, m. p. 54°.

The *cis* : *cis*-isomer was prepared as follows. *trans-n*-Non-5-enyl bromide was converted into *trans*-undeca-7-ene-1-yne (IX) by condensation with sodium acetylide, and the Grignard complex of the latter carboxylated at atmospheric pressure in ether with solid carbon dioxide. Catalytic semi-hydrogenation of the acetylenic linkage of the enyne acid (X) thus prepared yielded undeca-*cis*-1 : *trans*-7-diene-1-carboxylic acid (XI) (*S*-benzylthiuronium salt). The latter was converted into its chloride by oxalyl chloride under mild conditions. This was not purified but was converted directly into *N-iso*butylundeca-*cis*-1 : *trans*-7-diene-1-carboxyamide (II*a*) which proved to be a liquid. A second specimen was prepared by proceeding from the enyne acid (X) to its *iso*butylamide (XII) and then semi-hydrogenating the triple bond. The assumption that the catalysed semi-hydrogenation step yields an essentially *cis*-isomer is founded on an extensive literature to this effect (for summaries see Campbell and Campbell, *Chem. Reviews*, 1942, **31**, 77; Crombie, *Quart. Reviews*, 1952, **6**, 101). But there is no doubt that a small proportion of the *trans*-compound is often found, probably by the subsequent stereomutation on the catalyst of the *cis*-compound already formed (Farkas and Farkas, *Trans. Faraday Soc.*, 1937, **33**, 837). Its presence has been revealed by cryoscopic measurement and by Raman and infra-red spectroscopic measurement (Henne and Greenlee, *J. Amer. Chem. Soc.*, 1943, **65**, 2020; Sondheimer, *J.*, 1950, 877; G. Smets, *Acad. Roy. Belg., Classe Sci.*, 1947, **21**, 3). The level

of contamination doubtless varies with the conditions and speed of hydrogenation but the conditions used here have previously enabled *cis*-compounds to be synthesised and proved identical with natural materials (*e.g.*, Crombie and Harper, *J.*, 1950, 1152) without interference by *trans*-impurity.

Data for the undeca-1 : 7-diene-1-carboxylic acids and their derivatives are listed in Table 1. Small specimens of the *trans*-1 : *cis*-7- and *cis*-1 : *cis*-7-*iso*butylamides were generously made available by Drs. Raphael and Sondheimer and were used to obtain certain

TABLE 1. *Undecadiene-1-carboxylic acids and derivatives.*

| | B. p./mm. | $n_D^{20}$ | *S*-Bzthu,* m. p. | *N-iso*Butylamides B. p./mm. | $n_D^{20}$† | No. of C:C |
|---|---|---|---|---|---|---|
| *trans*-1 : *trans*-7- (I) ‡ ... | 137°/0·7 | 1·4734 [1] | 159° | 145°/4 × $10^{-3}$ | [m. p. 54°] | 1·95 |
| *cis*-1 : *trans*-7- (II) ‡ ... | 115—117°/0·5 | 1·4699 | 148 | 142°/3 × $10^{-2}$ | 1·4824 | 1·91 |
| *trans*-1 : *cis*-7- [2] (III) ‡ | 98—100°/$10^{-5}$ | 1·4710 | 143—144 | 133—135°/$10^{-5}$ | 1·4806 | — |
| *cis*-1 : *cis*-7- [3] (IV) ‡ ... | 108—109°/$10^{-4}$ | 1·4741 | 129—130 | 145—146°/$10^{-2}$ | 1·4830 | 1·81 [4] |

[1] M. p. 35°. [2] Data by Raphael and Sondheimer, *J.*, 1951, 2693. [3] Data by Raphael and Sondheimer, *J.*, 1950, 115. [4] New data obtained in the present work.
* *S*-Benzylthiuronium salt. † Adjusted to 20° by −0·0004 per degree within a range of ±5°.
‡ R=$CO_2H$.

TABLE 2. *Ultra-violet light absorption data.*[1]

| | $\lambda_{max.}$, mμ | $\varepsilon_{max.}$ |
|---|---|---|
| $Pr^n$·CH=CH·$[CH_2]_4$·CH=CH·CO·NHBu. | | |
| *trans*-1 : *trans*-7- (I*a*) | 226 | 11,500 |
| *cis*-1 : *trans*-7- (II*a*) | 226 | 10,700 |
| *trans*-1 : *cis*-7 (III*a*) | 227 | 10,500 |
| *cis*-1 : *cis*-7- (IV*a*) | 227 | 8,700 |
| *trans*-Me·$[CH_2]_6$·CH=CH·CO·NHBu[1] | 226 | 10,300 |
| *cis*- ,, ,, | 226 | 10,000 |
| Me·$[CH_2]_8$·CO·NHBu[1] | <215 | — |
| | (λ 215 | ε 1,000) |
| *trans*-Me·$[CH_2]_6$·CH=CH·$CO_2H$ | <215 | — |
| | λ 230 | ε 3,400 |
| | λ 215 | ε 11,900 |
| *trans*-Me·CH=CH·$CO_2H$ [2] | 205 | 15,700 |
| ,, Me·CH=CH·CO·$NH_2$ | <216 | — |
| | λ 216 | ε 12,000 |
| | λ 230 | ε 4,000 |
| ,, Me·CH=CH·CO·NHBu[1] | 227 | 8,500 |
| ,, Me·CH=CH·CO·$NEt_2$ [3] | 215 | 11,000 |

[1] Data obtained with ethanol as solvent. [2] C. J. Timmons, private communication. [3] Bowden Braude, and Jones, *J.*, 1946, 948.

new data recorded here. The carbon skeletons of the two stereoisomers prepared in the present work were demonstrated by hydrogenation of the diene acids to lauric acid.

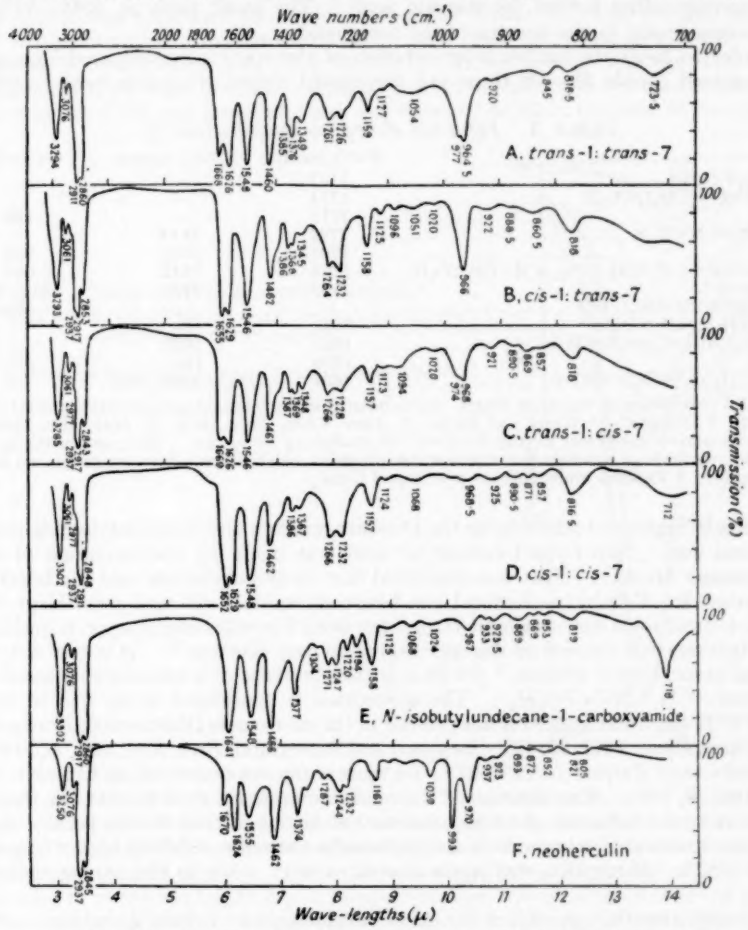
The melting points of the four *S*-benzylthiuronium salts of the undecadiene-1-carboxylic acids (Table 1) support the claim that they have the stereochemical structures assigned to them. These decrease as the bending of the chain increases [*i.e.*, m. p. of *trans*-1 : *trans*-7 (I*b*)>*cis*-1 : *trans*-7 (II*b*)>*trans*-1 : *cis*-7 (III*b*)>*cis*-1 : *cis*-7 (IV*b*)]. In a qualitative sense it is known that the more linear members of a comparable series of molecules pack more readily and therefore form more stable crystal lattices which have higher melting points.

The ultra-violet light absorption of all four stereoisomeric *iso*butylamides (Table 2) showed a maximum on a rising curve at 226 mμ. with an extinction coefficient of ~10,000. Such a maximum, whilst present in other αβ-unsaturated *iso*butylamides was absent in the saturated compounds. In the crotonic acid series it was noted that the *iso*butylamide maximum was at a wave-length longer than that of either the amide or the diethylamide. As regards geometrical isomerism, in such cases as the present where there is no steric hindrance to the planarity of the chromophore and where its length is the same in both the *cis*- and the *trans*-form (on the assumption that the preferred *s*-configuration about the intervening single bond is the same in both cases), current theory indicates that the ultra-violet light absorption of the isomers should be very similar. The variation in $\lambda_{max.}$ for the four stereoisomers is negligible (1 mμ) and, though the values of $\varepsilon_{max.}$ are not identical

within the limits of experimental error, it would be imprudent to draw conclusions on the present data alone, particularly since the low figure for the *cis* : *cis*-isomer may be associated with its low hydrogenation value (C:C, Table 1).

In order to gain further evidence of the discrete nature of the isomers and to support their assigned configurations the infra-red spectra of the *N-iso*butylundecadiene-1-carboxyamides have been examined (Fig. 1). The saturated isomer is included for comparison.

FIG. 1. *Infra-red spectra of* N-isobutylundeca-1 : 7-diene-1-carboxyamides *and related compounds.*




(*B*—*D*, liquid films; *A*, *E*, *F*, paraffin mulls, paraffin absorption present.)

The spectra, which are of pure liquid films or paraffin mulls of the material and are therefore influenced by intermolecular hydrogen bonding, show the usual features for monosubstituted amides. In the four stereoisomers the N–H stretching frequency is present at 3285—3302 cm.$^{-1}$, the amide band A (carbonyl) at 1626—1629 cm.$^{-1}$, and the amide band B—which is in the position characteristic of a monosubstituted amide—at 1546—1548 cm.$^{-1}$ (Richards and Thompson, *J.*, 1947, 1248; Randall, Fowler, Fuson, and Dangl, "Infra-Red Determination of Organic Structures," D. Van Nostrand, New York, 1949; Fuson, Josien, and Powell, *J. Amer. Chem. Soc.*, 1952, **74**, 1; and references cited in these works).

Conjugation displaces the carbonyl absorption to lower frequencies by some 10—15 cm.$^{-1}$ in the *iso*butylamides and the free acids (Table 3). In part as a consequence of the importance of the resonance form $R\cdot CH{=}CH\cdot C\overset{O^-}{\underset{\overset{+}{N}HBu^i}{<}}$ to the secondary amide structure (cf. Pauling, Corey, and Branson, *Proc. Nat. Acad. Sci.*, 1951, **37**, 205; *X*-ray crystallography indicates some 50% of double bond character for the carbon–nitrogen linkage, and the amide group is considered to be planar), the carbonyl frequency falls by some 70 cm.$^{-1}$ with respect to the corresponding ketone (or dimeric acid). The small peak at 3061—3076 cm.$^{-1}$ appears consistently in the spectra of the compounds.

In order to facilitate further interpretation of the spectra certain model compounds were examined (Table 3). Of those not mentioned above, *trans*-non-1-ene-1-carboxylic

TABLE 3. *Infra-red absorption maxima (cm.$^{-1}$).*

| | C=O, stretch | C=C, stretch | C=C—H bend |
|---|---|---|---|
| $Me\cdot[CH_2]_{10}\cdot CO_2H$ * | 1711 | — | — |
| *cis*-$Et\cdot CH{=}CH\cdot[CH_2]_2\cdot CO_2H$ | 1714 | — | — [1] |
| *trans*- ,, ,, | 1714 | — | 966·5 |
| *cis*-$Bu^n\cdot CH{=}CH\cdot CO_2H$ | 1702 | 1642 | — [1] |
| *trans*- ,, | 1702 | 1652 | 984 |
| *cis*-1:*trans*-7-$Pr^n\cdot CH{=}CH\cdot[CH_2]_4\cdot CH{=}CH\cdot CO_2H$ | 1698 | 1642 | 966 |
| *trans*-1 : *trans*-7- ,, ,, ,, * | 1700 | 1652 | 966·5, 979 [2] |
| *trans*-$Pr^n\cdot CH{=}CH\cdot[CH_2]_4\cdot OH$ | — | — | 966·5 |
| ,, $Me\cdot[CH_2]_{10}\cdot CO\cdot NHBu^t$ * | 1642 | — | — |
| *cis*-$Me\cdot[CH_2]_6\cdot CH{=}CH\cdot CO\cdot NHBu^t$ | 1631 | 1660 | — |
| *trans*- ,, * | 1629 | 1671 | 977 |
| ,, $Me\cdot[CH_2]_6\cdot C{\equiv}C\cdot CO\cdot NHBu^t$ | 1634 | 2257,[2] 2248, 2230 | — |

[1] Faint absorption at the wave-length characteristic of the *trans*-isomer (probably contamination, see text). [2] Shoulder. [3] Wotiz and Miller, *J. Amer. Chem. Soc.*, 1949, **71**, 3441, give 2190—2260 cm.$^{-1}$ (plus nearby satellites) for the R—C≡C—R stretching frequency. The complexity is said to be due to overtones or combination tones whose intensity has been enhanced by resonance with the fundamental. * Paraffin mull; others pure liquid films.

acid was made from *n*-octaldehyde by the Doebner reaction and its *iso*butylamide prepared in the usual way. Non-1-yne-1-carboxylic acid was made by carboxylation of non-1-ynylmagnesium bromide. This was converted into its *iso*butylamide and the latter semi-hydrogenated to *N-iso*butyl-*cis*-non-1-ene-1-carboxyamide. *cis*- and *trans*-Hept-2-enoic and -hept-4-enoic acids were available from other work (Crombie and Harper, unpublished).

Attention was first focused on the spectral region near 1000 cm.$^{-1}$. It is now well established that absorption at 966 cm.$^{-1}$ (10·35 μ) is characteristic of a *trans*-double bond in the environment $-CH_2\cdot CH{=}CH\cdot CH_2-$. The absorption is considered to be due to the unsaturated C–H deformation and is not present in the *cis*-isomers (Rasmussen, Brattain, and Zuco, *J. Chem. Phys.*, 1947, **15**, 135; Sheppard and Sutherland, *Proc. Roy. Soc., A*, 1949, **196**, 195; Crombie and Harper, *J.*, 1950, 873; for more extensive references see Crombie, *Quart. Reviews*, 1952, **6**, 101). Examination of the model compounds showed that the frequency was sensitive to the influence of conjugation on the carbon–carbon double bond. In both the *trans*-αβ-unsaturated acids and the *iso*butylamides there was a shift to higher frequencies of 12—18 cm.$^{-1}$. Absorption was again absent or very weak in the corresponding *cis*-isomers.

Considering now the spectra of the *N-iso*butylundeca-1 : 7-diene-1-carboxyamides in this region, the absorption at 977 and 965 cm.$^{-1}$ (Fig. 2) in the *trans*-1 : *trans*-7-isomer can be assigned to the C–H deformation frequency at the *trans*-1- and the *trans*-7-double bonds respectively. As expected, the *cis*-1 : *cis*-7-isomer has only weak absorption here and this is probably due to traces of stereoisomeric impurities. Similarly the *cis*-1 : *trans*-7-compound has only one absorption band and this at 966 cm.$^{-1}$ due to the *trans*-7-unsaturation. It would be predicted that the *trans*-1 : *cis*-7-diene *iso*butylamide should have but one absorption maximum in the C=C–H deformation region, and this at ~978 cm.$^{-1}$. The compound does in fact have a strong inflexion at 974 cm.$^{-1}$ but there is maximal absorption at 968 cm.$^{-1}$ (Fig. 2). This is interpreted as indicating the presence of some *trans*-stereoisomeric impurity which may have arisen during the semi-hydrogenation step involved in

its preparation * (Raphael and Sondheimer, *loc. cit.*). The position of the frequency corresponding with the C–H deformation about a *cis*-double bond is uncertain, though it is generally agreed to be lower than the *trans*. In this connection it may be noted that a band (818·5—816·5 cm.$^{-1}$) already present in the saturated and the *trans* : *trans*-compound is reinforced as one passes through the *cis* : *trans*- to the *cis* : *cis*-isomers.

In the C=C stretching region it was noted that the two diene *iso*butylamides with *cis*-1-double bonds has this vibration frequency at 1652 and 1655 cm.$^{-1}$ whilst the pair with *trans*-1-unsaturation showed it at 1668 and 1669 cm.$^{-1}$. Though small, the difference was quite definite and it was confirmed on model compounds (Table 3). Two further cases were located in the literature (Couvreuir and Bruylants, *Bull. Soc. chim. Belg.*, 1950, **59**, 436; G. S. Myers, *J. Amer. Chem. Soc.*, 1951, **73**, 2100). As can be seen from the table the shift only applies to the double bonds in conjugation with the carbonyl—at the cell thicknesses used (~5 μ) the C=C str. frequency was not detected at all in the case of *cis*- and *trans*-

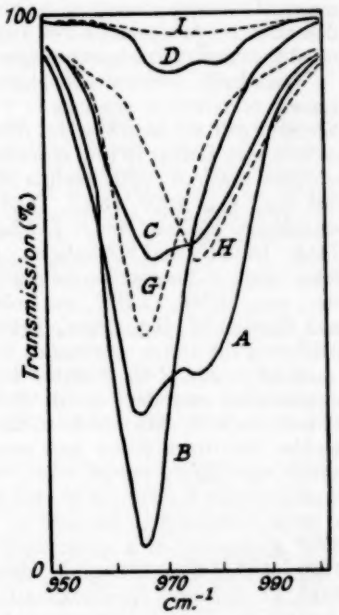



FIG. 2. *Unsaturated* C–H *deformation frequencies.*
(Detail from Fig. 1. *G—I* are for liquid films at qualitatively comparable thicknesses.)

*A*, *N-iso*-Butylundeca-*trans*-1 : *trans*-7-diene-1-carboxyamide.
*B*, ,, *cis*-1 : *trans*-7- ,, ,,
*C*, ,, *trans*-1 : *cis*-7- ,, ,,
*D*, ,, *cis*-1 : *cis*-7- ,, ,,
*G*, *trans*-*n*-non-4-en-1-ol.
*H*, *trans*-*N-iso*Butylundeca-1-ene-1-carboxyamide.
*I*, *cis*- ,, ,, ,,

hept-4-en-1-oic acids or in *trans*-non-5-en-1-ol. The reason is that the change in dipole moment during the C=C stretching vibration is small when the double bond is seated well within the carbon chain, but considerable when it is at the end of a chain or when a polar substituent is attached close by. Since the intensity of infra-red absorption is proportional (classically) to the square of the dipole moment change, the vibration is weak or not detectable in appropriate cases (Rasmussen, Brattain, and Zucco, *J. Chem. Phys.*, 1947, **15**, 135; Kletz and Sumner, *J.*, 1948, 1456; cf. Wotiz and Miller, *J. Amer. Chem. Soc.*, 1949, **71**, 3441). A frequency difference of the above type has long been recognised in the Raman spectra of geometrical isomers (Bourguel and Gredy, *Compt. rend.*, 1932, **195**, 129; Gredy and Piaux, *ibid.*, 1934, 198, 1235; Gredy, *Bull. Soc. chim.*, 1935, **2**, 1029; 1936, **3**, 1093, 1101; 1937, **4**, 415; *Compt. rend.*, 1936, **202**, 322; Goethals, *Bull. Soc. chim. Belg.*, 1937, **46**, 409; Ruzicka, Schinz, and Susz, *Helv. Chim. Acta*, 1944, **27**, 1561). In

* In support of this view *N-iso*butylnona-*trans*-1 : *cis*-5-diene-1-carboxyamide shows only one clean absorption at 978 cm.$^{-1}$. In this series, however, the two bands in the *trans*-1 : *trans*-5-isomer were not resolved although the shape of the absorption gives indication of their presence (Crombie, unpublished).

this case, however, the strength of the absorption depends on the polarisibility instead of on the dipole moment, and even double bonds in the interior of long carbon chains have strong Raman lines.

In view of the above evidence that the structures assigned by synthesis to the four stereoisomeric *N-iso*butylundeca-1 : 7-dienamides are correct (within the reservations mentioned regarding contamination with stereoisomers), their relation to natural herculin can now be considered. Jacobson (*J. Amer. Chem. Soc.*, 1948, **70**, 4234) records m. p. 59—60° for the natural material, so that on this basis only the *trans* : *trans*-synthetic compound might be identical though its m. p. is too low (53—54°). Furthermore, beyond a little yellowing, none of the synthetic *iso*butylamides deteriorated visibly on storage for months at room temperature whereas herculin is described as changing to a dark resin overnight. Their taste, although bitter and unpleasant, had none of the intensity and character of those natural *iso*butylamides which the present author has examined. Finally, when tested in acetone solution against adult *Tenebrio molitor* at concentrations of 1—2% all four geometrical isomers caused a mortality of less than 3%. Natural pellitorine which was described by Jacobson (*loc. cit.*) as being less toxic than herculin to houseflies caused a kill of 47% under the same conditions (actual concentration 1·17%).

Thus both chemical and physiological evidence indicate that herculin is not a stereoisomer of *N-iso*butylundeca-1 : 7-diene-1-carboxyamide. Since an original specimen of herculin was not available for direct comparison its re-isolation was undertaken. A procedure very similar to that described by Jacobson was followed. In the early stages of the isolation asarinin (xanthoxylum S) was obtained as colourless needles, m. p. 121° ($\lambda_{max.}$ 236, 286 mμ; $\varepsilon_{max.}$ 9600, 9600). The presence of this in southern prickly ash bark is well established (Colton, *Amer. J. Pharm.*, 1890, 191; Gordin, *J. Amer. Chem. Soc.*, 1906, **28**, 1649; Dieterle and Schwengler, *Arch. Pharm.*, 1939, **277**, 33; Jacobson, *loc. cit.*). At a later stage *N*-2-*p*-methoxyphenylethyl-*N*-methylcinnamamide, m. p. 75° ($\lambda_{max.}$ 223, 280 mμ; $\varepsilon_{max.}$ 21,500, 22,700), was isolated. This was first obtained from the bark by LaForge and Barthel (*J. Org. Chem.*, 1944, **9**, 250). Although asarinin is a pyrethrum synergist neither of the above compounds is insecticidally potent alone.

Finally, out of the fraction from which Jacobson isolated herculin a highly unstable unsaturated secondary amide (infra-red spectrum, Fig. 1), m. p. 63—65°, was isolated. It was markedly different from the stereoisomeric *N-iso*butylundeca-1 : 7-diene-1-carboxyamides described above and possessed the characteristic burning taste. The natural amide was highly potent when tested against houseflies (see Experimental) and a preliminary examination has shown it to be more highly unsaturated than is the diethenoid structure assigned to herculin by the American author. It has the typical ultra-violet light absorption of a conjugated triethenoid compound ($\lambda_{max.}$ 258, 270, 280 mμ, $E^{1\%}_{1\,cm.}$ 1290, 1680, 1490; O'Connor, Heinzelmann, Freeman, and Pack, *Ind. Eng. Chem. Anal.*, 1945, **17**, 467, give for elaeostearic acid: $\lambda_{max.}$ 260, 270, 281 mμ, $E^{1\%}_{1\,cm.}$ 1290, 1700, 1300, in *iso*octane). This polyeneamide is therefore named *neo*herculin and will be examined further. The nature of the herculin isolated by Jacobson must remain open.

## Experimental

The ultra-violet light absorption data (in pure ethanol) were obtained by Mrs. I. Boston, using a Hilger medium quartz instrument. Analytical data, including microhydrogenations which were determined in acetic acid with a platinum catalyst, are by Mr. Oliver of Imperial College.

The infra-red spectra were determined by the author using a Grubb–Parsons single beam spectrometer with rock-salt optics, a mechanical slit drive, and a Brown recorder. Subtraction of the atmospheric absorption on a point-to-point basis gave the curves illustrated. The error in reading the frequencies is believed to be ±15, ±2, and ±0·5 cm. at 3600, 1300, and 750 cm. respectively. Slit widths at 3850, 1300, and 750 cm. were 0·036, 0·20, and 0·88 mm. respectively. In the case of the model compounds (Table 3) only the regions of immediate interest were examined. The author is grateful to Dr. W. C. Price for the facilities afforded him in this phase of the investigation.

trans-n-*Oct*-4-*enyl Bromide*.—Phosphorus tribromide (117 g.) was added during 3 hours to a

stirred mixture of *trans*-oct-4-en-1-ol (150 g.; Crombie and Harper, *J.*, 1950, 1077) and dry pyridine (24 ml.), cooled in ice. The crude bromide was isolated by distillation at 150 mm., diluted with light petroleum (b. p. 40—60°), and washed with water, dilute hydrochloric acid (10%), and sodium hydroxide solution (10%). The petroleum layer was dried ($Na_2SO_4$) and evaporated and the residue distilled, to give trans-n-*oct*-4-*enyl bromide* (156 g., 70%), b. p. 78°/15·5 mm., $n_D^{20}$ 1·4682 (Found: C, 50·55; H, 7·85; Br, 41·3. $C_8H_{15}Br$ requires C, 50·3; H, 7·9; Br, 41·8%).

trans-n-*Oct*-4-*ene*-1-*carboxylic Acid* (V).—A Grignard reagent was prepared from *trans-n*-oct-4-enyl bromide (100 g.) and magnesium turnings (12·7 g.) in anhydrous ether (400 ml.). Dry gaseous carbon dioxide was circulated above the vigorously stirred solution until the mixture became very viscous. A large excess of crushed solid carbon dioxide was then stirred into the paste (by hand), and the mixture set aside overnight. The product was decomposed with water and dilute hydrochloric acid and extracted with ether. The ethereal extract was itself thoroughly extracted with dilute sodium hydroxide solution, and the latter washed with more ether. The purified acid was liberated by acidification (hydrochloric acid) to Congo-red and collected with ether. Working up in the usual way and distillation gave trans-n-*oct*-4-*ene*-1-*carboxylic acid* (59·2 g., 73%), b. p. 138—140°/15·5 mm., $n_D^{20}$ 1·4460 (Found: 69·3; H, 10·65. $C_9H_{16}O_2$ requires C, 69·2; H, 10·3%). The S-*benzylthiuronium* salt formed shining plates (from ethyl acetate), m. p. 145—146° (Found: C, 63·0; H, 7·95; N, 8·55. $C_{17}H_{26}O_2N_2S$ requires C, 63·3; H, 8·15; N, 8·7%).

trans-n-*Non*-5-*en*-1-*ol* (VI).—*trans-n*-Oct-4-ene-1-carboxylic acid (22·4 g.) in dry (calcium hydride) ether (100 c.c.) was added slowly (1 hour) to a solution of lithium aluminium hydride (5·2 g.) in dry ether (150 ml.). The ether refluxed gently. When addition was complete stirring was continued for 1 hour and the mixture decomposed with water and then dilute hydrochloric acid. Drying ($Na_2SO_4$), evaporation, and distillation yielded trans-n-*non*-5-*en*-1-*ol* (20·4 g., 93%), b. p. 107°/17·5 mm., $n_D^{20}$ 1·4475 (Found: C, 75·7; H, 12·5. $C_9H_{18}O$ requires C, 76·0; H, 12·75%). The p-*diphenylurethane* formed long colourless needles, m. p. 102°, from light petroleum (b. p. 60—80°) (Found: C, 78·65; H, 8·3. $C_{22}H_{27}O_2N$ requires C, 78·3; H, 8·05%).

The infra-red spectrum showed strong absorption at 966·5 cm.$^{-1}$, confirming the presence of a *trans*-double bond.

trans-n-*Non*-5-*enyl Bromide*.—A mixture of *trans-n*-non-5-en-1-ol (34 g.) and pyridine (6 ml.), when treated as above with phosphorus tribromide (24 g.), yielded trans-n-*non*-5-*enyl bromide* (40·5 g., 82·5%), b. p. 143—146°/90 mm., $n_D^{20}$ 1·4690 (Found: Br, 38·4. $C_9H_{17}Br$ requires Br, 38·95%).

trans-n-*Dec*-6-*enal* (VII).—A Grignard reagent was prepared from *trans-n*-nonenyl bromide (15 g.) and magnesium turnings (1·9 g.) in dry ether (25 ml.) and heated under reflux for 1 hour and then cooled to 20°. Ethyl orthoformate (11 g.) was added and the mixture refluxed for 16 hours (solid began to separate after ~15 minutes). The ether was removed by distillation and the residue decomposed with dilute hydrochloric acid (150 ml.; 4%). The aldehyde was removed by distillation in steam and isolated from the distillate (230 ml.) as its bisulphite compound (colourless plates). Addition of the latter to an excess of dilute sodium hydrogen carbonate solution, steam-distillation, and extraction with ether gave the pure aldehyde. The ethereal solution was dried, the solvent evaporated (this caused some difficulty as a stable froth formed), and the residue twice distilled, to yield *trans-n*-dec-6-enal (3·7 g., 38%), b. p. 117°/50 mm., $n_D^{20}$ 1·4409. Only a poor analysis was obtained (Found: C, 76·3; H, 11·6. Calc. for $C_{10}H_{18}O$: C, 77·85; H, 11·75%). The aldehyde had a characteristic odour, quite pleasant in bulk but powerful on dilution, and opinions as to its pleasantness or unpleasantness in this state varied. It formed a 2:4-*dinitrophenylhydrazone* which crystallised from hot ethanol first as golden-yellow flat needles, but these were followed on further cooling by red prisms. By careful crystallisations from methanol the yellow needles were isolated; they had m. p. 93° (Found: C, 57·1; H, 6·6; N, 17·5. $C_{16}H_{22}O_4N_4$ requires C, 57·4; H, 6·6; N, 16·85%).

n-*Undeca*-trans-1: trans-7-*diene*-1-*carboxylic Acid* (VIII).—*trans-n*-Dec-6-enal (2·4 g.) was added slowly to a mixture of malonic acid (1·66 g.) and pyridine (1·7 ml.) and set aside in a closed vessel with a capillary leak, with occasional shaking ($CO_2$ evolved) for 5 days. All the solid had then disappeared and the product was warmed for 2 hours on the steam-bath. To the cooled liquid excess of dilute hydrochloric acid was added and the neutral plus acid material extracted with ether. The acid was purified by extraction and by liberation from 10% sodium hydroxide solution in the usual way. It was collected with ether, and the solution dried ($Na_2SO_4$), evaporated, and distilled. The n-*undeca*-trans-1: trans-7-*diene*-1-*carboxylic acid*

(1·38 g., 45%), b. p. 140°/0·8 mm., $n_D^{20}$ 1·4737, crystallised in large colourless plates. The crystals melted at 34—35° to a turbid liquid which cleared at 36° (Found : C, 73·7; H, 10·2. $C_{12}H_{20}O_2$ requires C, 73·45; H. 10·3%). Microhydrogenation : 2·04 $H_2$. The acid had a mild odour. Its S-*benzylthiuronium salt* crystallised in shining plates (from ethyl acetate), m. p. 159° (Found : N, 7·45. $C_{10}H_{30}O_2N_2S$ requires N, 7·75%). A specimen of the acid (96 mg.) was hydrogenated in ethyl acetate in presence of palladium on calcium carbonate until no more gas was absorbed. Filtration and evaporation yielded lauric acid, m. p. 41° alone and on admixture with a specimen, m. p. 41°. Its *p*-bromophenacyl ester melted at 74·5° (lit., 76°).

N-iso*Butyl*-n-*undeca*-trans-1 : trans-7-*diene*-1-*carboxyamide* (I).—Thionyl chloride (0·3 ml.) was added to the *trans* : *trans*-acid prepared as above (554 mg.), set aside for 12 hours, and then heated at 100° for 30 minutes. The crude acid chloride was diluted with benzene (5 ml.), and excess of *iso*butylamine (0·7 ml.) added dropwise with cooling to moderate the vigorous reaction. The product was set aside for 30 minutes and then washed with 10% hydrochloric acid, 10% aqueous sodium hydroxide, and water. The benzene solution was dried and evaporated, and the residue distilled, having b. p. 145°/4 × $10^{-3}$ (600 mg.); it rapidly crystallised to a straw-yellow mass of needles, m. p. 46—47°, clear at 51°. The material proved difficult to crystallise but colourless needles, obtained from light petroleum (b. p. 40—60°), had m. p. 53—54°. For analytical and other purposes the solution in light petroleum was treated with charcoal and kieselguhr and filtered and the filtrate evaporated. The residue (faintly yellow only) was dried at 70°/0·2 mm. for 3 hours to remove all traces of solvent and melted at 53°, clear at 54·5° (Found : C, 76·05; H, 11·75; N, 5·25. $C_{16}H_{29}ON$ requires C, 76·45; H, 11·65; N, 5·5%). Microhydrogenation : 1·95 $H_2$. For the light absorption see Table 2.

trans-n-*Undeca*-7-*en*-1-*yne* (IX).—Sodamide was prepared in liquid ammonia (200 ml.) from sodium (2·5 g.) and a ferric nitrate catalyst. Scrubbed ($H_2SO_4$) acetylene was passed in to form sodium acetylide in the usual way and *trans*-*n*-non-5-enyl bromide (15 g.) added in ether (10 ml.). The flask was lagged and the mixture was stirred for 18 hours. Excess of ammonium chloride was then added and the ammonia evaporated off on a steam-bath. The residue was treated with dilute sulphuric acid, and the acetylenic hydrocarbon isolated by ether-extraction. This solution was worked up in the usual way to give, after two distillations, trans-n-*undeca*-7-*en*-1-*yne* (6·8 g., 62%), b. p. 82—84°/18 mm., $n_D^{20}$ 1·4462 (Found : C, 88·1; H, 12·1. $C_{11}H_{18}$ requires C, 87·9; H, 12·1%). Although it gave an immediate white precipitate with alcoholic silver nitrate, yet when treated with Johnson and McEwen's potassiomercuric iodide reagent (*J. Amer. Chem. Soc.*, 1926, **48**, 469) only a white turbidity was obtained which after a few minutes became a turbid green, a small amount of crystalline material separating.

trans-n-*Undeca*-7-*en*-1-*yne*-1-*carboxylic Acid* (X).—Ethylmagnesium bromide was prepared in dry ether (100 c.c.) from magnesium (1·5 g.) and ethyl bromide (6·8 g.). *trans*-*n*-Undeca-7-en-1-yne (6·0 g.) in ether (20 c.c.) was added slowly and the mixture refluxed for 3 hours. The acetylenic Grignard solution was then poured carefully on a large excess of crushed solid carbon dioxide and set aside overnight. An excess of dilute hydrochloric acid was added and the ethereal phase separated. The acid was extracted with dilute sodium hydroxide solution and purified and worked up in the usual way. Distillation gave pure trans-n-*undeca*-7-*en*-1-*yne*-1-*carboxylic acid* (3·4 g., 44%), b. p. 125—126°/0·04 mm., $n_D^{20}$ 1·4745 (Found : C, 74·55; H, 9·7. $C_{12}H_{18}O_2$ requires C, 74·2; H, 9·35%). Microhydrogenation : 2·96 $H_2$. The S-*benzylthiuronium* salt of the acid crystallised in needles (from ethyl acetate), m. p. 180° (Found : N, 7·45. $C_{20}H_{28}O_2N_2S$ requires N, 7·75%).

n-*Undeca*-cis-1 : trans-7-*diene*-1-*carboxylic Acid* (IX).—*trans*-*n*-Undeca-7-en-1-yne-1-carboxylic acid (1·93 g.) was hydrogenated in methyl acetate (15 ml.) with pre-reduced palladium on calcium carbonate (5%; 150 mg.) until hydrogen equivalent to one mol. (224 ml. at N.T.P.) had been absorbed. Kieselguhr was added and the catalyst filtered off. The solvent was evaporated and the residue distilled, to give n-*undeca*-cis-1 : trans-7-*diene*-1-*carboxylic acid* (1·35 g.), b. p. 115—117°/0·5 mm., $n_D^{20}$ 1·4682—1·4693. Microhydrogenation : 1·80 $H_2$. The acid was refractionated and then had b. p. 127—129°/1·0—1·1 mm., $n_D^{20}$ 1·4692—1·4699 (Found : C, 73·15; H, 10·5. $C_{12}H_{20}O_2$ requires C, 73·45; H, 10·3%). Microhydrogenation : 1·80 $H_2$. Its S-*benzylthiuronium* salt (Found : N, 7·55%) when crystallised from ethyl acetate formed shining plates, m. p. 148°. The derivative melted quite sharply, but repeated re-determinations showed that, for this and certain other *S*-benzylthiuronium salts mentioned, the value obtained was not always precisely repeatable : variations of a few degrees on either side of the m. p. quoted were obtained. Other derivatives would seem preferable for characterisation but these salts were used in order to effect a comparison with the isomers recorded in the literature.

The *cis*-1 : *trans*-7-acid (101 mg.) was hydrogenated, as described for the *trans*-1 : *trans*-7-acid and yielded lauric acid, m. p. 41° alone and on admixture with a commercial specimen.

trans-N-iso*Butyl*-n-*undeca*-7-*en*-1-*yne*-1-*carboxyamide* (XII).—The acetylenic acid (522 mg.) was converted into the acid chloride with thionyl chloride (0·3 ml.) during 2 hours at 20° and 1 hour at 60°. This was diluted dry benzene (5 ml.), and excess of *iso*butylamine added to the chilled solution. Working up as described for the *iso*butylamide above gave trans-N-iso*butyl*-n-*undeca*-7-*en*-1-*yne*-1-*carboxyamide* (509 mg.), b. p. 159—160°/0·2 mm., $n_D^{20}$ 1·4854 (Found: N, 5·4. $C_{16}H_{27}ON$ requires N, 5·6%).

N-iso*Butyl*-n-*undeca*-cis-1 : trans-7-*diene*-1-*carboxyamide*.—(*a*) *trans*-*N*-*iso*Butyl-*n*-undeca-7-en-1-yne-1-carboxyamide (409 mg.) was hydrogenated in ethyl acetate (8 ml.) with pre-reduced palladium on calcium carbonate (5%; 100 mg.). After semihydrogenation (36·8 ml. at N.T.P.), the *iso*butylamide was isolated in the usual way. Distillation yielded N-iso*butyl*-n-*undeca*-cis-1 : trans-7-*diene*-1-*carboxyamide*, b. p. 154°/0·6 mm., 142°/3 × $10^{-3}$ mm., $n_D^{20}$ 1·4824. Microhydrogenation: 1·91 $H_2$.

(*b*) The *cis*-1 : *trans*-7-acid (0·585 g.) was refluxed with oxalyl chloride (1·0 ml.) in anhydrous benzene (5 ml.) for 1 hour and the solvent and excess of reagent were removed *in vacuo* at 20°. Ether (20 ml.) and excess of *iso*butylamine (1·0 ml.) were added and the mixture was set aside for 1 hour. Water was added, and the ethereal layer separated and worked up as described for the *trans*-1 : *trans*-7-isomer. Distillation gave *N*-*iso*butyl-*n*-undeca-*cis*-1 : *trans*-7-diene-1-carboxyamide, b. p. 128—130°/4 × $10^{-3}$ mm., $n_D^{18}$ 1·4795 (Found: C, 76·4; H, 11·95; N, 5·25%). Microhydrogenation: 1·88 $H_2$.

*Non*-1-*yne*.—*n*-Heptyl bromide (25·7 g.) was added to sodium acetylide (prepared from sodium, 4·0 g., with an iron catalyst for the formation of sodamide) in liquid ammonia (300 ml.) and stirred overnight. The ammonia was evaporated off, water and ether were added, and the nonyne (9·7 g., 53%) was isolated in the usual way; it had b. p. 149—152°, $n_D^{21}$ 1·4261 (Bourguel, *Ann. Chim.*, 1925, **3**, 359, gives b. p. 149°, $n_D^{20}$ 1·423, and Truchet, *ibid.*, 1931, **16**, 410, gives b. p. 149—151°, $n_D^{20}$ 1·426, for specimens prepared by the isomerisation of non-2-yne).

*Non*-1-*yne*-1-*carboxylic Acid*.—A Grignard reagent was prepared from ethyl bromide (8·2 g.) and magnesium (1·8 g.) in ether. Non-1-yne (6·2 g.) was added and the mixture refluxed for 6 hours, poured on a large excess of solid carbon dioxide, and sealed for 36 hours in an autoclave. The product was decomposed with dilute hydrochloric acid, and the acid extracted with ether and separated from neutral products by dilute aqueous sodium hydroxide. Liberation of the acetylenic acid with a slight excess of dilute hydrochloric acid (Congo-red), extraction with ether, and distillation furnished non-1-yne-1-carboxylic acid (4·2 g., 50%), b. p. 110—111°/0·3 mm., $n_D^{18}$ 1·4617 (Found: C, 71·0; H, 9·55. Calc. for $C_{10}H_{16}O_2$: C, 71·4; H, 9·6%) (Moureu and Delange, *Compt. rend.*, 1903, **136**, 554, *Bull. Soc. chim.*, 1903, **29**, 660, give b. p. 164—168°/18—20 mm.).

N-iso*Butyl*-n-*non*-1-*yne*-1-*carboxyamide*.—Thionyl chloride (1·3 g.) was added to non-1-yne-1-carboxylic acid (1·3 g.) and set aside overnight. After 30 minutes' refluxing the crude acid chloride was diluted with ether and cooled in ice, and *iso*butylamine (3 ml.) in ether (10 ml. was added dropwise. After washing with acid and alkali and distillation, the required acetylenic iso*butylamide* (1·48 g., 82%), b. p. 130—132°/5 × $10^{-4}$ mm., $n_D^{17}$ 1·4752, was isolated (Found: N, 5·7. $C_{14}H_{25}ON$ requires N, 6·25%).

N-iso*Butyl*-cis-n-*non*-1-*ene*-1-*carboxyamide*.—The acetylenic *iso*butylamide (0·974 g.) was hydrogenated in ethyl acetate (5 ml.) with pre-reduced palladium (5%) on calcium carbonate until 90 c.c. of hydrogen at 20°/758 mm. (1 mol., 105 ml.) had been absorbed. The catalyst was filtered off and the solution evaporated and distilled, one fraction, b. p. 121°/2 × $10^{-4}$ mm., $n_D^{21}$ 1·4704 (0·752 g.), being collected. This was redistilled and the middle fraction b. p. 128°/9 × $10^4$ mm., $n_D^{22}$ 1·4689, used for spectroscopic work and for analysis (Found: N, 5·8. $C_{14}H_{27}ON$ requires N, 6·2%). Microhydrogenation: 1·08 $H_2$.

trans-n-*Non*-1-*ene*-1-*carboxylic Acid*.—*n*-Octaldehyde (9·6 g.; b. p. 172—175, $n_D^{22}$ 1·4170) was added to a cooled mixture of pyridine (8 ml.) and malonic acid (7·8 g.) and set aside at room temperature until all solid had disappeared (3 days with occasional shaking). It was then heated on a steam-bath for 2 hours, diluted with water, and acidified, and the acidic and neutral materials were isolated with ether. The acid was purified by extraction with aqueous sodium hydroxide (10%) and isolated in the usual way. Distillation gave *trans*-non-1-ene-1-carboxylic acid (6·5 g., 51%), b. p. 113—114°/0·3 mm., $n_D^{15}$ 1·4630 (Found: C, 70·05; H, 10·65. $C_{10}H_{18}O_2$ requires C, 70·55; H, 10·65%). Zaar (*Ber. Schimmel and Co., A.-G.*, 1929, 299; *Chem. Abs.*, 1930, **24**, 2107) gives b. p. 148—149°/4·5 mm., $n_D^{20}$ 1·4616.

N-iso*Butyl*-trans-n-*non*-1-*ene*-1-*carboxyamide*.—The *trans*-acid (1·87 g.) when converted into

the crude acid chloride with thionyl chloride and added to an excess of *iso*butylamine afforded the required N-iso*butylamide* (2·07 g.), b. p. 140°/8 × $10^{-4}$ mm., which rapidly solidified to waxy needles, m. p. 48—49° (Found: C, 74·5; H, 12·3; N, 5·85. $C_{14}H_{27}ON$ requires C, 74·6; H, 12·1; N, 6·2%). Microhydrogenation: 0·98 $H_2$.

N-iso*Butyl*-trans-*prop*-1-*ene*-1-*carboxyamide* (Crotono*iso*butylamide).—Prepared in the usual way this formed sheaves of needles, m. p. 71°, from light petroleum (b. p. 40—60°) (Found: C, 67·55; H, 10·2. Calc. for $C_8H_{15}ON$: C, 68·05; H, 10·7; N, 9·9%).

*Examination of Southern Prickly Ash Bark.*—The bark was a gift from Messrs. S. B. Penick, New York, which was received through the good offices of Dr. S. H. Harper. Ground bark (5 kg.) was macerated under nitrogen with light petroleum (b. p. 40—60°) for 12 days and the extract syphoned off. It was then macerated with four further portions (5 l. each) of light petroleum and the united extracts were evaporated to 2·5 l. This solution was successively extracted with one portion of 280 ml., three of 225 ml., and two of 100 ml. of nitromethane. The nitromethane was removed *in vacuo* and the residue dissolved in ether (400 ml.) and washed with hydrochloric acid (10%), sodium hydroxide solution (10%), and water. The aqueous alkali tended to form emulsions.

When the purified ethereal solution was set aside at 0° for 24 hours solid was deposited which crystallised from ethanol as long colourless needles, m. p. 121°, $[\alpha]_D^{21}$ −125° (*c*, 2·90%, chloroform) (Found: C, 67·9; H, 5·35. Calc. for $C_{20}H_{18}O_6$: C, 67·8; H, 5·1%). Huang-Minlon (*Ber.*, 1937, **70**, 951) gives m. p. 121—122°, $[\alpha]_D^{18}$ −122° (*c*, 3·54%, chloroform), for asarinin. For the ultra-violet light absorption, see p. 3000. G. Aulin Erdtmann and H. Erdtmann (*Svensk Papperstidn.*, 1944, **47**, 22) record $\lambda_{max.}$ 237, 287 mμ; $\varepsilon_{max.}$ 9300, 8300. The green-brown solution was evaporated *in vacuo* and the residue extracted with boiling light petroleum (b. p. 60—80°; 10 × 100 ml.). A brown syrup remained undissolved, and oil separated from the hot solutions which were concentrated to 400 ml. and then decanted; the solution was set aside at 0° for 36 hours. Crystals (1·4 g.), m. p. 58—72°, were deposited. After four recrystallisations from light petroleum (b. p. 40—60°) the material (colourless needles) melted at 75°. For ultra-violet light absorption, see p. 3000. LaForge and Barthel (*loc. cit.*) give m. p. 76° for *N*-2-*p*-methoxyphenylethyl-*N*-methylcinnamamide.

Further concentration of the main solution to 120 c.c. and chilling at 0° for 36 hours caused no more crystallisation, so the solution was cooled to −50° for 30 minutes. A gel (A) separated which was filtered off. The filtrate was further concentrated and set aside at 0° for 3 weeks but only a small amount of crystallisation was induced. Finally, it was evaporated *in vauco* and used for insecticidal tests as below. Recrystallisation of (A) (which formed an amorphous pad on filtration) from light petroleum (b. p. 60—80°) with charcoal and kieselguhr and cooling to 0° gave crude neo*herculin*, m. p. 57—58° (2·0 g.). A portion was twice recrystallised as above, washed with cold light petroleum (b. p. 40—60°), and dried at 0·05 mm. It formed colourless needles, m. p. 63—65° (Found: N, 5·25. $C_{16}H_{25}ON$ requires N, 5·65%). Jacobson (*loc. cit.*) gives m. p. 59—60° for herculin. The hydrogenation number was 65·7. For ultra-violet light absorption, see p. 3000. Although unstable at room temperature (and, though less so, at 0°) in the presence of air, a specimen sealed *in vacuo* has been preserved at 0° for more than a year without deterioration.

*neo*Herculin in odourless petroleum distillate containing 15% of *cyclo*hexanone was tested against houseflies by Dr. E. A. Parkin of the Pest Infestation Laboratory (D.S.I.R.), Slough. It was 4·2 times as toxic as the oily mother-liquors (see above) and the concentration equivalent to a 0·1% (w/v) solution of standard natural pyrethrins was between 0·13 and 0·18% (24 hours' mortality count). Although showing some knock-down effect (10 minutes), neither of the samples was as good as 0·1% pyrethrins, even at 1·0% strength.

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## 575. *Cinnolines. Part XXIX.* 8-Hydroxycinnoline and Some of its Derivatives.*

By E. J. Alford, H. Irving, (Miss) H. S. Marsh, and K. Schofield.

8-Hydroxy- and 8-methoxy-cinnoline have been synthesised, together with a number of 4-substituted derivatives. The absorption spectra of 8-hydroxy- and 8-hydroxy-4-methyl-cinnoline in acid, neutral, and alkaline solutions have been measured and compared with those of 8-methoxycinnoline and corresponding compounds of the quinoline series. The acidic and basic dissociation constants of the 8-hydroxycinnolines are compared with those of the 8-hydroxyquinolines, and discussed in relation to their respective bacteriostatic actions and abilities to chelate with metals. The quantitative dibromination of 8-hydroxycinnoline has been used to determine the solubility of the compound.

8-Hydroxyquinoline ("oxine") (XVII) forms chelated complexes with a large number of metals, and many attempts have been made to improve its selectivity as an analytical reagent by the introduction of substituents or by annelation (Irving, Butler, and Ring, *J.*, 1949, 1489, and refs. therein). However, apart from the effect of 2-substituents which prevent the formation of insoluble tris-complexes with aluminium, possibly for steric reasons, no instances of exceptional behaviour have been reported. Derivatives of oxine have also been used extensively in medicine, and the relation between the bacteriostatic action of compounds of this type and the strength of the complexes they form with metals of biological interest has been thoroughly studied (Albert *et al.*, *Biochem. J.*, 1947, **41**, 529, 534; *Brit. J. Exp. Path.*, 1945, **26**, 160; 1947, **28**, 69).

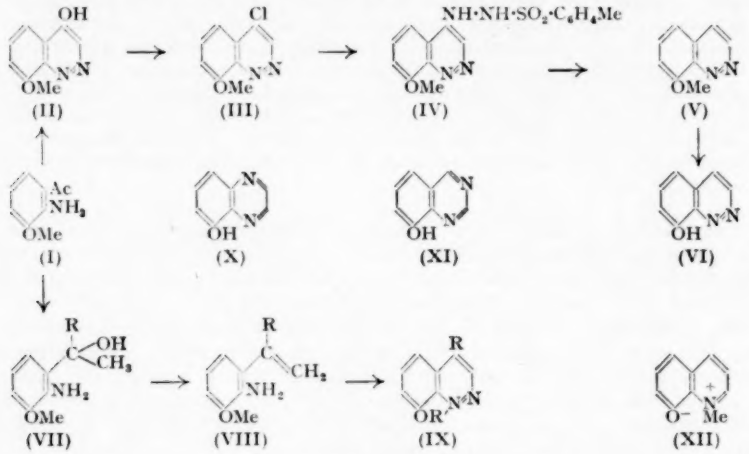
The significance of the acidic and basic dissociation constants of ampholytes such as oxine for interpretations of their antibacterial activity and their role in analytical chemistry is now beginning to be appreciated, and a more extensive knowledge of the capacities to form metal complexes and related properties of other heterocyclic systems which incorporate the characteristic chelating group of oxine would clearly be of interest. To this end we have begun an investigation of some analogues in which a methine group is replaced by a nitrogen atom. Of the three possible isomers this paper deals with the preparation and properties of 8-hydroxycinnoline (VI) and some of its derivatives. Preliminary measurements on 8-hydroxyquinoxaline (X) have been carried out by Freeman and Spoerri (*J. Org. Chem.*, 1951, **16**, 438), and our results for this reagent and for 8-hydroxyquinazoline (XI) will be communicated later.

*Preparation of 8-Hydroxycinnoline and its Derivatives.*—Synthesis of 8-hydroxycinnoline presented an interesting problem. 2-Amino-3-methoxyacetophenone, a compound of fully authenticated structure, was readily available (Simpson, Atkinson, Schofield, and Stephenson, *J.*, 1945, 646), but previous attempts (Schofield and Simpson, *ibid.*, p. 520) to convert it into 4-hydroxy-8-methoxycinnoline (I ⟶ II) by diazotisation in dilute acid failed. Later work on this type of cyclisation (*idem*, *J.*, 1948, 1170) clearly demonstrated that its success depended largely upon enolisation of the ketone group, an acid-catalysed process. Accordingly (I) was now diazotised in concentrated hydrochloric acid, whereupon it gave 92% of (II). Comments on the large-scale preparation of (I) will be found in the Experimental section.

Other experiments in the cinnoline series (Alford and Schofield, to be published) made it probable that removal of the hydroxyl group from (II) would be possible by converting the latter into 4-chloro-8-methoxycinnoline (III), which would then react with toluene-*p*-sulphonylhydrazide giving (IV), whence 8-methoxycinnoline (V) would arise by alkaline decomposition. In fact each of these steps proceeded without difficulty, and 8-methoxycinnoline was readily demethylated by hydrobromic acid to 8-hydroxycinnoline (VI). The overall yield of (VI) from *m*-nitroacetophenone (10 steps) was 7·4%, or from *m*-hydroxyacetophenone (8 steps) 16·6%.

* Part XXVIII, *J.*, 1952, 2102.

2-Amino-3-methoxyacetophenone is a useful intermediate for the synthesis of 4-substituted 8-hydroxycinnolines. With methylmagnesium iodide and phenylmagnesium bromide it gave the alcohols (VII; R = Me or Ph), which were readily dehydrated. Diazotisation of the ethylenes (VIII; R = Me or Ph) produced 8-methoxy-4-methyl- (IX; R = R′ = Me) and 8-methoxy-4-phenyl-cinnoline (IX; R = Ph, R′ = Me), each of which was converted by hydrobromic acid into the 8-hydroxy-compound (IX; R = Me or Ph, R′ = H).




8-Methoxycinnoline, which formed an orange picrate, was a colourless crystalline solid which quickly liquefied and became green in the air. Cinnoline itself behaves in the same way (Morley, *J.*, 1951, 1971). In contrast, 8-methoxy-4-methyl- and 8-methoxy-4-phenyl-cinnoline were orange and yellow respectively. The former was stable, but the latter slowly became sticky in the air.

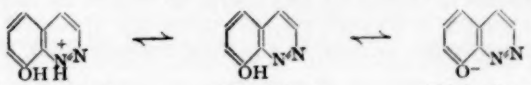
8-Hydroxycinnoline melted at a higher temperature than the two 4-substituted derivatives, and also much higher than 8-hydroxyquinoline. Whilst the latter is colourless, 8-hydroxycinnoline was bright yellow. 8-Hydroxy-4-methyl- and 8-hydroxy-4-phenyl-cinnoline were light yellow and fawn-coloured, respectively.

8-Hydroxycinnoline formed an orange-red hydrobromide, and the reddish-orange solution of the compound in sodium hydroxide solution deposited a sodium salt on cooling. A crimson dye was formed by coupling with benzenediazonium chloride. Woroshtzow and Kogan (*Ber.*, 1932, **65**, 142) converted oxine " almost quantitatively " into 8-aminoquinoline by means of the Bucherer reaction and we have found conditions which gave 87% of 8-aminocinnoline (isolated as its acetyl derivative) from the hydroxy-compound by this method.

8-Aminocinnoline, a yellow crystalline solid obtained by hydrolysis of its acetyl derivative, gave deep purple solutions in dilute acid, and formed a deeply coloured picrate: Woroshtzow and Kogan (*loc. cit.*) noted that acid solutions of 8-aminoquinoline were deep red. 8-Hydroxycinnoline dissolved slowly in ethereal diazomethane to the accompaniment of brilliant colour changes, and formed a very dark blue product which could not be purified. It is likely that this substance possesses a structure similar to that of the brick-red product obtained by this method from oxine, for which the expression (XII) has been proposed (Caronna and Sansone, *Gazzetta*, 1939, **69**, 24; H. and M. Schenkel-Rudin, *Helv. Chim. Acta*, 1944, **28**, 1456).

*Absorption Spectra of 8-Hydroxycinnolines.*—The absorption spectrum of 8-methoxycinnoline in 95% methyl alcohol is extremely similar to that of 8-hydroxycinnoline, but is displaced slightly towards shorter wave-lengths while the subsidiary maximum at 315 mμ is lacking. The spectrum of the methoxy-compound in 0·01N-sodium hydroxide is virtually unchanged in position, and the apparent decrease in molecular extinction coefficient shown

in Table 1 is certainly not a real effect but reflects only the difficulty of handling this very unstable substance. Ionisation of the phenolic group of 8-hydroxycinnoline in 0·01N-sodium hydroxide produces the anion (XIV) and there is a pronounced bathochromic shift of 11, 27, and 65 mμ for the prominent ultra-violet, medium, and long-wave peaks respec-



(XIII; $H_2Cnx^+$) (VI; HCnx) (XIV; $Cnx^-$)

tively. On solution in 0·01N-hydrochloric acid, the formation of the cation (XIII) again causes a bathochromic shift in the spectrum of 8-hydroxycinnoline amounting to 7, 1, and 70 mμ for the corresponding peaks. The behaviour of the methoxy-compound (V) is almost identical. Table 1 summarises the salient features of these spectra which show remarkable analogies to those of 8-hydroxy- and 8-methoxy-quinoline (Ewing and Steck, *J. Amer. Chem. Soc.*, 1946, **68**, 2181) although they are displaced considerably towards longer wave-lengths, as might have been expected from the introduction of the grouping –N:N– in place of –N:CH–. Phillips, Huber, Chung, and Merritt (*ibid.*, 1951, **73**, 630) have discussed the absorption spectra of the 8-hydroxyquinolines in terms of the structures (XV) to (XVIII) and point out correctly that the inability of 8-methoxyquinolines to undergo comparable structural changes on passing from a neutral to a basic medium explains the virtual identity of their absorption spectra in *cyclo*hexane and aqueous sodium hydroxide. They do not, however, comment on the fact that 8-methoxyquinoline cannot undergo structural changes comparable to (XVII ⟶ XVI ⟶ XV), and we suggest that the close similarity of the absorption spectra of 8-methoxy- and -hydroxy-quinoline in an acidic solvent is strong evidence against the presence in appreciable quantity of the keto-form (XV). The occurrence of this keto-form, first postulated on chemical grounds

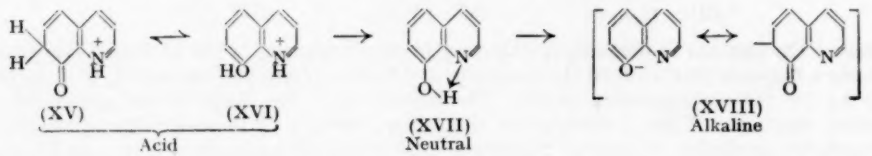
TABLE 1. *Absorption spectra of 8-hydroxycinnoline and its derivatives.*

| *In* 0·01N-HCl | | *In* 95% *methanol* | | *In* 0·01N-NaOH | |
|---|---|---|---|---|---|
| λ (mμ) | log ε (mol.) | λ (mμ) | log ε (mol.) | λ (mμ) | log ε (mol.) |
| *8-Hydroxycinnoline.* | | | | | |
| — | — | — | — | 240 (min.) | 3·95 |
| 252 (max.) | 4·62 | 245 (max.) | 4·53 | 256 (max.) | 4·40 |
| 289 | 3·00 | 270 | 2·67 | 290 | 2·74 |
| 304 (max.) | 3·07 | 303 (max.) | 3·03 | 330 (max.) | 3·43 |
| — | — | 306 | 3·00 | — | — |
| — | — | 315 (max.) | 3·04 | — | — |
| 335 | 2·63 | 320 | 3·00 | 359 | 2·94 |
| 430 (max.) | 3·32 | 360 (max.) | 3·43 | 425 (max.) | 3·49 |
| *8-Methoxycinnoline.* | | | | | |
| 247 (max.) | 4·34 | 242 (max.) | 4·64 | 242 (max.) | 4·38 |
| 290 | 3·06 | 261 | 3·05 | 270 | 2·99 |
| 300 (max.) | 3·08 | 295 (max.) | 3·32 | 295 (max.) | 3·12 |
| 328 | 2·74 | 310 | 3·25 | 310 | 3·00 |
| 422 (max.) | 3·28 | 350 (max.) | 3·64 | 358 (max.) | 3·44 |
| *8-Hydroxy-4-methylcinnoline.* | | | | | |
| 250 (max.) | 4·38 | 246 (max.) | 4·42 | 255 (max.) | 4·40 |
| 290 | 3·04 | 268 | 2·68 | 287 | 2·76 |
| 308 (max.) | 3·14 | 310 (max.) | 3·09 | 330 (max.) | 3·46 |
| 334 | 2·46 | 320 | 3·05 | 355 | 3·00 |
| 420 (max.) | 3·43 | 360 (max.) | 3·50 | 415 (max.) | 3·60 |

by Stone and Friedman (*ibid.*, 1947, **69**, 209) and supported by magnetic-susceptibility measurements (Seguin, *Bull. Soc. chim.*, 1946, **13**, 566), is not confirmed by infra-red studies of oxine and its derivatives (Phillips, *J. Amer. Chem. Soc.*, 1949, **71**, 3984), or by our present measurements in the related cinnoline series. The success of the Bucherer reaction in both series might have been counted as support for the existence of the keto-forms according to one theory of the mechanism of this reaction (*Org. Reactions*, **1**, 105; but see Cowdrey, *J.*, 1946, 1046).

The formation of metal chelates of 8-hydroxycinnoline (see below) may be taken to indicate the probability of intramolecular hydrogen bonding in the neutral molecule (Hunter, *Ann. Reports*, 1946, **43**, 141). No such bonding is, however, possible in the 8-methoxy-derivative, and the close similarity of the absorption spectra of 8-hydroxy- and 8-methoxy-cinnoline in neutral solvents affords no support for this hypothesis.

The absorption spectra of 8-hydroxy-4-methylcinnoline in neutral, acid, and alkaline media show the same features as those of 8-hydroxycinnoline itself, but the introduction




of the 4-methyl group causes a slight hypsochromic shift (Table 1) of the peak of longest wave-length and an increase in the intensity of absorption of the ionised forms. The same effect has been noted by Phillips (*loc. cit.*) and Phillips and Merritt (*J. Amer. Chem. Soc.*, 1952, **74**, 552) among 4-methyl-oxines for which comparative data are given in Table 2.

TABLE 2.

| | *In* 0·01N-HCl | | *In* 95% MeOH | | *In* 0·01N-NaOH | |
|---|---|---|---|---|---|---|
| | $\lambda_{max.}$ | log $\varepsilon_{max.}$ | $\lambda_{max.}$ | log $\varepsilon_{max.}$ | $\lambda_{max.}$ | log $\varepsilon_{max.}$ |
| 8-Hydroxycinnoline | 430 | 3·32 | 360 | 3·43 | 425 | 3·49 |
| 8-Hydroxy-4-methylcinnoline | 420 | 3·43 | 360 | 3·50 | 415 | 3·60 |
| 8-Hydroxyquinoline | 358 | 3·23 | 320 | 3·38 | 345 | 3·43 |
| 8-Hydroxy-4-methylquinoline | 350—353 | 3·38 | 319 | 3·52 | 343 | 3·59 |

*Dissociation Constants of 8-Hydroxy- and 8-Hydroxy-4-methyl-cinnoline.*—Owing to the general similarity in the absorption spectra of 8-hydroxycinnoline in acid and alkali solutions, change of pH produces less marked alterations in the spectra of its aqueous solutions than is often the case in acid–base systems. Nevertheless, by careful selection of wave-lengths it is possible to determine its dissociation constants by the method described by Irving, Ewart, and Wilson (*J.*, 1949, 2672) for oxine. Values for the Brønsted dissociation constants defined by $K_1' = [H^+][HCnx]/[H_2Cnx^+]$ and $K_2' = [H^+][Cnx^-]/[HCnx]$ obtained from spectrophotometric measurements on a series of buffer solutions of constant ionic strength (0·3M), covering the pH range 2—10, are given in Table 3. Assuming that the

TABLE 3.

| Wave-length (mμ) | 300 | 330 | 320 | 370 | 410 | Mean value |
|---|---|---|---|---|---|---|
| 8-Hydroxycinnoline | | | | | | |
| $pK_1'$ | 3·15 | — | 2·95 | — | 3·05 | 3·05 |
| $pK_2'$ | 8·01 | — | 8·00 | — | 7·80 | 7·94 |
| 8-Hydroxy-4-methylcinnoline | | | | | | |
| $pK_1'$ | — | 3·50 | — | 3·45 | 3·45 | 3·47 |
| $pK_2'$ | — | 8·55 | — | 8·45 | 8·45 | 8·48 |

activity coefficient $f_{HCnx}$ does not differ appreciably from unity, while $f_{H_2Cnx^+}$ (or $f_{Cnx^-}$) is equal to the mean activity coefficient of a typical univalent ion of radius 2·5 Å, at the same ionic strength, we obtain $\log f_{\pm} = -0{\cdot}19$ whence the thermodynamic dissociation constants of the species $H_2Cnx^+$ (XIII) and HCnx (VI) at 14°±3° are given by $pK_1 = 2{\cdot}86 \pm 0{\cdot}06$ and $pK_2 = 8{\cdot}13 \pm 0{\cdot}07$. Potentiometric titration at 25° of $7{\cdot}6 \times 10^{-3}$M-8-hydroxycinnoline solutions in 0·036M-perchloric acid with carbonate-free 1·25N-alkali gave the values 2·90 and 8·30. Spectrophotometric measurements with 8-hydroxy-4-methylcinnoline gave Brønsted dissociation exponents of 3·47 and 8·48 (Table 3) for $\mu = 0{\cdot}3$, whence $pK_1 = 3{\cdot}28 \pm 0{\cdot}02$ and $pK_2 = 8{\cdot}67 \pm 0{\cdot}04$ at 14°±3°.

## Experimental

(A temperature given in parentheses before a melting point refers to the characteristic temperature of sintering.)

Unless otherwise stated, extracts were dried with anhydrous sodium sulphate.

m-*Hydroxyacetophenone*.—By the method of Simpson *et al.* (*loc. cit.*), except that the acid copper sulphate solution was boiled during the addition of the diazonium solution, *m*-aminoacetophenone (54 g.) gave the phenol (30 g., 56%), m. p. 94—96°.

m-*Methoxyacetophenone*.—*m*-Hydroxyacetophenone (175 g.), water (1750 c.c.), and half of a solution of sodium hydroxide (87 g.) in water (500 c.c.) were stirred on the steam-bath whilst the remaining alkali and methyl sulphate (175 c.c.) were added dropwise during 5 minutes. After a further ½ hour's heating the product was extracted with ether. Distillation gave a colourless oil (161 g., 83%), b. p. 130—132°/15 mm.

3-*Methoxy*-2-*nitroacetophenone*.—Small-scale experiments indicated that the heating at the end of the nitration recommended by Simpson *et al.* (*loc. cit.*) caused only insignificant improvement in yield. More satisfactory was the use of longer nitration periods. *m*-Methoxyacetophenone (60 g.) was added rapidly (there was no appreciable evolution of heat) to nitric acid (360 c.c.; *d* 1·42) stirred at 0—5°, and the solution was set aside for 54 hours at room temperature. The product (68·8 g.) was precipitated by water, and crystallised from alcohol, giving 3-methoxy-2-nitroacetophenone (34·2 g., 44%), m. p. 129—130°. The acid liquor slowly deposited colourless needles (m. p. 42—45°), which could not be purified by crystallisation. When reduced in the way described for the 2-nitro-compound this material gave an oily solid which benzoylation converted into a product which could be separated by crystallisation from methanol and mechanical sorting into yellow prisms, m. p. (105°) 111—116°, and soft white needles, m. p. (129°) 133—136°. Sufficient of these compounds was not obtained for complete purification, but the former was very probably impure 6-benzamido-3-methoxyacetophenone, which forms yellow prisms, m. p. 117—118° (Simpson *et al.*, *loc. cit.*).

2-*Amino*-3-*methoxyacetophenone*.—The powdered nitro-ketone (36 g.) was added in portions during 50 minutes to a stirred suspension of iron filings (52 g.), water (193 c.c.), and acetic acid (193 c.c.), at 75—80°. The mixture was then refluxed and stirred for 1 hour, and, after cooling, extracted with ether. Evaporation of the solvent after drying ($Na_2CO_3$), and recrystallisation of the residue from alcohol gave the amine (28 g., 92%) as large yellow crystals, m. p. 64—66°.

4-*Hydroxy*-8-*methoxycinnoline*.—Powdered 2-amino-3-methoxyacetophenone (20 g.) in hydrochloric acid (*d* 1·16; 1 l.) was diazotised at 0—5° by sodium nitrite (10 g.) in water (20 c.c.). After the addition of further concentrated hydrochloric acid (2 l.) the solution was left overnight at room temperature and then heated at 60° until it no longer gave a coupling reaction (*ca.* 6 hours). Evaporation under reduced pressure on the steam-bath, dissolution of the residue in water (250 c.c.), and neutralisation with anhydrous sodium acetate, followed by boiling (charcoal) and filtration, gave a crystalline product (19·7 g., 92%), m. p. 162—164·5°. 4-*Hydroxy*-8-*methoxycinnoline* gave white needles of its *monohydrate*, m. p. 164—165° (Found: C, 55·8; H, 5·4. $C_9H_8O_2N_2,H_2O$ requires C, 55·7; H, 5·2%), after crystallisation from water and drying at 110°/2 mm.

4-*Chloro*-8-*methoxycinnoline*.—The 4-hydroxy-compound (15 g.) and phosphorus oxychloride (60 c.c.) were heated for ¼ hour at 95°, and the solution was then decomposed with ice and basified with sodium hydroxide solution. Extraction with ether and removal of the solvent gave clusters of faint yellow needles (14·4 g., 87%), m. p. 140—142°. 4-*Chloro*-8-*methoxycinnoline* crystallised from acetone in long, thick, light yellow needles, m. p. 142—143° (Found: C, 55·6; H, 3·5. $C_9H_7ON_2Cl$ requires C, 55·6; H, 3·6%), which slowly darkened.

By standard methods the chloro-compound gave 8-*methoxy*-4-*phenoxycinnoline*, which formed stout colourless needles or plates, m. p. 123—124° (Found: C, 71·7; H, 4·6. $C_{15}H_{12}O_2N_2$ requires C, 71·4; H, 4·8%), from aqueous acetone.

8-*Methoxy*-4-N′-(*toluene*-p-*sulphonyl*)*hydrazinocinnoline Hydrochloride*.—Toluene-*p*-sulphonylhydrazide (4·5 g.) in warm chloroform (125 c.c.) was treated with 4-chloro-8-methoxycinnoline (2·4 g.) in the same solvent (10 c.c.). After the solution had been kept overnight, scratching initiated the separation of an orange solid, which was collected after 1 week [4·4 g.; m. p. 176—179° (decomp.)]. Crystallisation from acetic acid–chloroform gave yellowish-orange crystals of 8-*methoxy*-4-N′-(*toluene*-p-*sulphonyl*)*hydrazinocinnoline hydrochloride*, m. p. 169—172° (decomp.) (Found: C, 47·7; H, 4·75. $C_{16}H_{17}O_3N_4ClS,H_2O$ requires C, 48·2; H, 4·8%). In experiments using 40 g. of the chloro-compound the yield reached 96%.

8-*Methoxycinnoline*.—The powdered toluenesulphonyl compound (19·5 g.) was added to sodium carbonate (195 g.; anhydrous) and water (1950 c.c.) at 95°, and the mixture was then refluxed for ½ hour, cooled, filtered, and extracted with chloroform. The extract provided a dark brown oil (8·1 g.) which readily solidified and was suitable for demethylation. Distillation gave a yellow oil (6·8 g.; b. p. 144—145°/0·3—0·4 mm.) which crystallised. 8-*Methoxycinnoline* formed, from benzene–ligroin (b. p. 60—80°), colourless needles, m. p. (in a sealed tube) 67—70·5° (Found: C, 67·2; H, 5·2. $C_9H_8ON_2$ requires C, 67·5; H, 5·0%), which quickly liquefied, darkened, and became green when kept. The *picrate* formed orange needles, m. p. (183°) 189—191·5° (with darkening) (Found: C, 46·5; H, 2·45. $C_9H_8ON_2,C_6H_3O_7N_3$ requires C, 46·3; H, 2·85%).

8-*Hydroxycinnoline*.—The methoxy-compound (4·4 g. of crude material) was refluxed with hydrobromic acid (*d* 1·5; 40 c.c.) for 2 hours. The solution was cooled rapidly to produce a finely divided precipitate (slow cooling gave clusters of large orange needles of the hydrobromide), and neutralised with ammonia whilst being cooled in ice. The yellow precipitate (2·8 g.; m. p. 175—185°) of 8-*hydroxycinnoline* crystallised from benzene in long yellow needles, m. p. (177°) 185—186° (Found: C, 65·5; H, 4·2; N, 19·9. $C_8H_6ON_2$ requires C, 65·75; H, 4·1; N, 19·2%). A little more of the substance was obtained by extracting the neutral filtrate with chloroform.

2-(2-*Amino*-3-*methoxyphenyl*)*propan*-2-*ol*.—2-Amino-3-methoxyacetophenone (10 g.) in dry ether (100 c.c.) was added slowly to a Grignard reagent [from magnesium (5·2 g.), ether (125 c.c.), and methyl iodide (15 c.c.)], and the mixture was refluxed for ½ hour and then decomposed with ice and ammonium chloride. Extraction with ether gave a solid (10·9 g.; m. p. 76—77·5°). The *alcohol* formed glistening leaflets, m. p. 78—79·5° (Found: C, 66·2; H, 8·1. $C_{10}H_{13}O_2N$ requires C, 66·3; H, 8·3%), from ether–light petroleum (b. p. 40—60°).

2-(2-*Amino*-3-*methoxyphenyl*)*propylene*.—The alcohol (2·5 g.), benzene (25 c.c.), and phosphoric oxide (5 g.) were refluxed for 3 hours. The mixture was decomposed with ice and ammonia and extracted with ether. Removal of the solvent gave an oil (2·3 g.) which was used in the next stage. The *benzamido*-derivative, prepared from the oil in a little acetone by treatment with benzoyl chloride and aqueous sodium hydroxide, crystallised from alcohol as white prisms, m. p. 206—206·5° (Found: C, 76·6; H, 6·35. $C_{17}H_{17}O_2N$ requires C, 76·4; H, 6·4%).

8-*Methoxy*-4-*methylcinnoline*.—The propylene (6 g.) in sulphuric acid (2·7 c.c. of concentrated acid and 24 c.c. of water) was diazotised with sodium nitrite (2·6 g.) in water (5·5 c.c.) at 0—5°. The solution was diluted with iced water (400 c.c.) and set aside in the dark for 4 days at room temperature, and then neutralised with sodium hydroxide solution (it became green), and extracted with chloroform. The dark red residue remaining on removal of the solvent gave dark red needles (4·3 g.), m. p. 123—129°, when crystallised from benzene–light petroleum (b. p. 60—80°). Pure 8-*methoxy*-4-*methylcinnoline* formed orange needles, m. p. 130—132° (Found: C, 68·5; H, 6·0. $C_{10}H_{10}ON_2$ requires C, 68·95; H, 5·8%).

8-*Hydroxy*-4-*methylcinnnoline*.—The above compound (crude, 4·2 g.) was heated under reflux with hydrobromic acid (*d* 1·5; 40 c.c.) for 2 hours, and the solution was then neutralised with aqueous ammonia whilst being cooled, and was then extracted with chloroform. The extract on evaporation gave a dark residue which was crystallised from benzene (charcoal) and gave a product (2·1 g.), m. p. 172—174°. Pure 8-*hydroxy*-4-*methylcinnoline* separated from benzene as light yellow prisms, m. p. 177—178·5° (Found: C, 67·9; H, 5·3. $C_9H_8ON_2$ requires C, 67·5; H, 5·0%).

1-(2-*Amino*-3-*methoxyphenyl*)-1-*phenylethyl Alcohol*.—Prepared from 2-amino-3-methoxyacetophenone (5 g.) by the use of phenylmagnesium bromide (4 equivalents), as with the methyl compound, this *alcohol* (6·3 g.; m. p. 93—95°, after one crystallisation) formed clusters of slightly impure chunky prisms, m. p. 94—95° (Found: C, 75·3; H, 6·6. $C_{15}H_{17}O_2N$ requires C, 74·0; H, 7·05%), from light petroleum (b. p. 60—80°).

α-(2-*Amino*-3-*methoxyphenyl*)*styrene*.—The alcohol (2 g.), when dehydrated as above, gave the crude oily ethylene (1·7 g.), suitable for use in the next stage. The *benzamido*-derivative crystallised from aqueous alcohol in clusters of colourless needles, m. p. 99—100·5° (Found: C, 80·0; H, 5·75. $C_{22}H_{19}O_2N$ requires C, 80·2; H, 5·8%).

8-*Methoxy*-4-*phenylcinnoline*.—The ethylene (1 g.) in sulphuric acid (0·33 c.c. of concentrated acid in 3 c.c. of water) was diazotised with sodium nitrite (0·32 g.) in water (0·7 c.c.) at 0—5°. Iced water (45 c.c.) was added and the solution was left 3 days at room temperature before being basified with sodium hydroxide solution and extracted with chloroform. Evaporation of the extract gave an oil (1·4 g.) which slowly solidified and crystallised from chloroform–light petroleum (b. p. 60—80°) as yellow plates (0·9 g.), m. p. 65—70°. Further crystallisation gave

8-methoxy-4-phenylcinnoline as opaque, light yellow plates, m. p. (78°) 85—89°. The compound became sticky in air, and a satisfactory specimen for analysis could not be obtained.

*8-Hydroxy-4-phenylcinnoline.*—The methoxy-compound (0·8 g.) was heated under reflux with hydrobromic acid (*d* 1·5; 8 c.c.) for 2 hours, and worked up as before. The tarry red solid which separated solidified overnight and was then extracted with boiling light petroleum (b. p. 60—80°). Evaporation of the extract yielded dull yellow prisms (0·37 g.), m. p. 136—140°. *8-Hydroxy-4-phenylcinnoline* formed clusters of fawn-coloured needles, m. p. 142—143·5° (Found: C, 75·9; H, 4·7. $C_{14}H_{10}ON_2$ requires C, 75·7; H, 4·5%), from benzene–light petroleum.

*Reactions of 8-Hydroxycinnoline.*—(a) *With diazomethane.* When 8-hydroxycinnoline (0·5 g.) was added to an ethereal solution of diazomethane (from 1 c.c. of nitrosomethylurethane) it slowly dissolved with effervescence, producing a brilliant emerald-green colour which changed to deep blue. The dark blue precipitate (0·35 g.), m. p. *ca.* 119—121°, which separated overnight could not be crystallised. It was insoluble in cold water, but dissolved slightly on boiling to give a red-brown solution. The solid dissolved readily in dilute mineral acids giving yellow solutions, and addition of alkali caused darkening and precipitation of a solid.

(b) *The Bucherer reaction.* The cinnoline (0·5 g.), aqueous ammonia (*d* 0·88; 7 c.c.), and ammonium sulphite (7 c.c. of a saturated solution) were heated in a sealed tube at 100—120° for 17 hours. The product was diluted with water, treated with aqueous sodium hydroxide, and extracted with ether. Removal of the solvent left a black oil (0·42 g.) which was dissolved in boiling acetic anhydride (6 c.c.), and the solution was then heated at 95° for 1 hour. The solution was decomposed with water (charcoal), filtered, and extracted with ether. The residue from the dried ($K_2CO_3$) extract formed yellow crystals (0·42 g.), m. p. 171—174°, from benzene–light petroleum (b. p. 60—80°). Further crystallisation gave 8-acetamidocinnoline as yellow prisms, m. p. 173—175° (Found: C, 63·8; H, 4·8. Calc. for $C_{10}H_9ON_3$: C, 64·2; H, 4·85%). Morley (*loc. cit.*) gave m. p. 177—178°. Higher reaction temperatures lowered the yield and produced much tar.

8-Acetamidocinnoline (0·3 g.) and hydrochloric acid (5N; 7 c.c.) were refluxed for 40 minutes, and the deep purple solution was then cooled in ice and basified with sodium hydroxide solution. The colour was discharged, and golden-yellow crystals separated. *8-Aminocinnoline* crystallised from benzene–light petroleum as golden-yellow needles, m. p. 89—92° (Found: C, 62·2; H, 5·4. $C_8H_7N_3,\frac{1}{2}H_2O$ requires C, 62·3; H, 5·2%). The *picrate* crystallised from acetic acid, first as fluffy dark brown needles, and later as hard jet-black needles, m. p. (230°) 236—238° (Found: C, 45·0; H, 2·9. $C_8H_7N_3,C_6H_3O_7N_3$ requires C, 44·9; H, 2·7%).

(c) *Reactions with metals.* Sensitivity tests in buffers of pH 5, 8, and 12 were carried out as described by Irving, Butler, and Ring (*loc. cit.*), and stability constants were measured in 73 : 27 dioxan–water, the Bjerrum–Calvin technique of potentiometric titration being used. Full details will be reported elsewhere.

Absorption spectra were measured with a Beckman Model DU Spectrophotometer, with minimum slit widths. " 95% Methyl alcohol " was prepared by adding redistilled water (5 ml.) to methyl alcohol (95 ml.). " AnalaR " reagents were used with water redistilled from an all-Pyrex apparatus in making up solutions, and every precaution was taken to prevent the intrusion of metallic impurities. Using 1-cm. matched silica cells it was shown that Beer's law was obeyed by 8-hydroxycinnoline and its ions at 370 and 410 mμ for concentrations up to at least 36 mg./l., and by 4-methyl-8-hydroxycinnoline and its ions at 294, 370, and 470 mμ up to at least 120 mg./l. The actual concentrations used varied from $10^{-3}$ to $10^{-5}$M, according to the part of the spectrum under examination. Buffer solutions prepared from sodium acetate and hydrochloric acid, potassium dihydrogen and disodium hydrogen phosphate, borax and hydrochloric acid, or sodium carbonate and hydrochloric acid, were made up with sufficient sodium chloride to give a constant ionic strength of 0·3M. The appropriate buffer containing no organic solute was used in the comparison cell, and measurements of pH were made to 0·01 unit with a Cambridge pH meter.

Solubility measurements were carried out by shaking mechanically in large stoppered Pyrex test-tubes at room temperature (i) distilled water and excess of 8-hydroxycinnoline, and (ii) a solution saturated with solute at the b. p. and chilled to produce fine crystals. At intervals portions of the clear solution were filtered through sintered glass, diluted 10-fold, and treated (10 ml.) in turn with hydrochloric acid (2N; 15 ml.), potassium bromide (1 g.), and potassium bromate (0·00253M; 2·0 ml.). After 5 minutes, potassium iodide solution (10%; 10 ml.) was added and liberated iodine was titrated with sodium thiosulphate (0·00987N). Preliminary measurements with known weights of 8-hydroxycinnoline showed that quantitative dibromin-

ation was achieved under these conditions. After 6 and 12 hours' shaking the concentration of hydroxycinnoline in (i) was 0·500 and 0·612 g./l., showing that solution was proceeding slowly, but consistent figures of 0·778 and 0·778 g./l. in (ii) after the same lapse of time showed the absence of supersaturation when equilibrium was approached from this side.

## Discussion

The introduction of a second nitrogen atom into a six-membered ring already containing one nitrogen atom greatly reduces the basic strength (Albert, Goldacre, and Phillips, *J.*, 1948, 2240), the p$K$ values of the acids conjugate to quinoline, cinnoline, quinazoline, and quinoxaline being 4·94, 2·7, 3·5, and *ca.* 0·8 respectively. The basic strengths of the corresponding 8-hydroxy-derivatives follow the same pattern, values for p$K_1$ being 5·00, 2·86, 3·41, and 2·15, while values for p$K_2$ of 9·85, 8·13, 8·65, and 8·73 respectively reflect the increasing tendency of the phenolic hydrogen atom to dissociate. It is noteworthy that the acid strengths of the corresponding 4-hydroxy-quinoline, -cinnoline, and -quinazoline, follow the same sequence since in 50% aqueous alcohol p$K_1$ is 2·41, 1·77, and 2·07, and p$K_2$ 12·43, 9·53, and 9·98 respectively (Keneford, Morley, Simpson, and Wright, *J.*, 1949, 1356). With the exception of quinoxaline these relative orders are those predictable from the electron densities on $N_{(1)}$ calculated by Longuet-Higgins and Coulson (*J.*, 1949, 971; cf. Keneford, Morley, Simpson, and Wright, *J.*, 1950, 1104) who discuss similar discrepancies in the case of pyridine, quinoline, and acridine (*loc. cit.*, p. 979).

In so far as it is possible to correlate the stability of a metal complex with the dissociation constants of the ligand (cf. Bjerrum, *Chem. Reviews*, 1950, **46**, 381; Irving, Discussion on Co-ordination Chemistry, 1951, I.C.I. Report No. BRL/149) we might expect 8-hydroxycinnoline to act like 8-hydroxyquinoxaline, especially at high pH, and for both of these reagents to chelate much less strongly with metals than does oxine itself. The stability constants of magnesium, nickel, zinc, and copper 8-hydroxycinnoline complexes in 73% aqueous dioxan (Irving and Marsh, to be published shortly) are found to be less than those of the corresponding oxine complexes, and the results of sensitivity tests, some of which are given in Table 4, confirm the general prediction. A full account of the interaction of

Table 4.

| Metal used : | | $Cu^{++}$ | $Zn^{++}$ | $Fe^{+++}$ | $Al^{+++}$ | $Mn^{++}$ | $Co^{++}$ |
|---|---|---|---|---|---|---|---|
| 8-Hydroxyquinoline [a] | A | 1·6 | 1·0 | 1·0 | 3·0 | 3·0 | 3·0 |
| | B | 2·0 | 4·0 | 4·0 | 6·0 | N.P. | 18 |
| | C | 3·7 | 7·0 | N.P. | N.P. | ,, | 18 |
| 8-Hydroxycinnoline [b] | A | N.P.* | N.P.* | 6·6 | N.P.* | N.P. | 71 |
| | B | ,, | ,, | N.P. | ,, | ,, | 19 |
| | C | ,, | ,, | ,, | ,, | ,, | N.P. |
| 8-Hydroxyquinoxaline [c] | A | 4·0 | 6·5 | N.P.§ | N.P. | N.P. | 3·0 |
| | B | ,, | ,, | ,, | ,, | 5·5 | 4·0 |
| | C | N.P. | N.P. | ,, | ,, | 700 | N.P. |

[a] Berg, "Die Analytische Verwendung von *o*-Oxychinolin," Stuttgart, 1938; the slight difference n buffer compositions does not affect the general conclusions. [b] Present authors. [c] Freeman and Spoerri (*loc. cit.*).

Sensitivity limits are given in μg./ml. and represent the lowest concentrations of metals to give visible precipitates under the conditions of test. The letters A, B, and C refer to buffers of pH 5·2, 8·4, and 12·4 (Irving, Butler, and Ring, *loc. cit.*). N.P. signifies that there was no precipitation of complex. The symbol § signifies no precipitation up to 1000 μg./ml.

* Under conditions of potentiometric titration with more concentrated solutions, insoluble aluminium, zinc, and copper complexes are precipitated.

metals with 8-hydroxycinnoline will be published elsewhere, but of the metals studied the only ones with which it shows a greater sensitivity than oxine are Hg(II) and Ga (but not In) in buffer A, Hg(II) and In (but not Ga) in buffer B, and Pd in buffer C. Apart from reactions of moderate sensitivity with Cu(I), Ag, Ru, and Rh in buffer A, and with Cu(I) and Pt in buffer B, the substance gave no evidence of potential value in analytical chemistry. As expected the low stability of its metal complexes is reflected in the relative activity of 8-hydroxy-quinoline and -cinnoline towards micro-organisms (Table 5).

The favourable effect of a 4-substituent, revealed by tests *in vitro*, has not yet been correlated with factors such as the lipoid solubility and stability of the metal complexes; but it is a striking fact that all three hydroxycinnolines proved to be inactive when mice

infected with hæmolytic streptococci, *P. berglei*, or *T. equiperdum* were treated intraperitoneally with the maximum tolerated dose (2·0, 2·0, and 5·0 mg./20-g. mouse respectively) once daily for 4 days.

After this work had been completed we learned that Albert and Hampton (to whom we are indebted for the dissociation constants of 8-hydroxyquinazoline) had undertaken and

TABLE 5. *Bacteriostatic indices.*

| | Hæmolytic streptococcus | *Staph. aureus* | *B. coli* |
|---|---|---|---|
| 8-Hydroxyquinoline [a] | — | 100,000 | 1,600 |
| 8-Hydroxycinnoline [b] | 7,300 | 730 | 290 |
| 8-Hydroxy-4-methylcinnoline [b] | 29,200 | 2,900 | 730 |
| 8-Hydroxyquinoxaline [c] | — | 1,200 | — |
| 8-Hydroxy-4-phenylcinnoline [b] | 111,000 * | 44,400 * | § |

[a] Albert, Rubbo, Goldacre, and Balfour, *Brit. J. Exp. Path.*, 1947, **28**, 69. [b] Data provided by the National Institute for Medical Research, for Hedley Wright broth after 12 hours' incubation at 37°. [c] Freeman and Spoerri (*loc. cit.*). * The figures are only approximate as the compound is so slightly soluble. § The compound was not inhibitory in saturated solution at pH 7·5, the solubility being approx. 10 mg./l.

completed the synthesis of all six possible 8-hydroxyazaquinolines, and were engaged in studies of their bacterial action and ability to form complexes with metals. Their route to 8-hydroxycinnoline differs from ours, and they have generously suggested the independent publication of results.

Grateful acknowledgement is made to the Department of Scientific and Industrial Research for the loan of apparatus, to the Council of University College, Exeter, for financial assistance, and to Drs. A. T. Fuller, F. Hawking, and J. Thurston of the National Institute for Medical Research for the bacteriostatic results.

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## 576. *Silica Sols. Part I. The Titration of Silica Sols.*

By K. C. BRYANT.

The effect of particle size and electrolyte concentration on the titration curve of alkaline silica sols has been investigated, the pH being depressed by an increase in electrolyte concentration and by a decrease in particle size. The results obtained are compared with those obtained with polymeric acids by other workers. The qualitative difference is explained by the dissolution of the silica particles at high pH, and the effects observed are interpreted as a consequence of the preferential adsorption of cations.

DESPITE the numerous investigations of the titration of silica sols (see Mukherjee and Chatterjee, *Nature*, 1945, **155**, 85, for references) the influence of particle size and electrolyte content on the titration curve appears not to have been studied. Evidence is presented here to show that these factors exert a considerable effect, and the results have been examined in the light of recent theories of the titration of polymeric electrolytes.

### EXPERIMENTAL

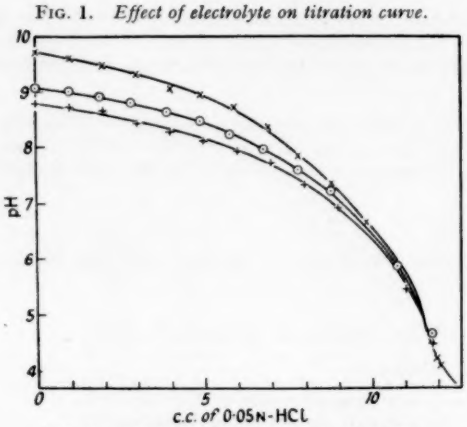
(a) *Preparation of Silica Sols.*—These were of three types, A, B, and C, differentiated chiefly by their particle size, which was governed by the method of preparation. The A type sol was produced by acidifying sodium silicate solution with hydrochloric acid until the pH had fallen to a low value, and allowing the reaction mixture to gel. After ageing, and removal of electrolyte by washing of the gel with water, the product was treated with dilute sodium hydroxide and dispersed by autoclaving. Lumps of undispersed gel were removed by centrifugation. The final product was a white mobile liquid, resembling diluted milk. It contained about 15% of silica (w/w), with a $SiO_2 : Na_2O$ ratio of about 250, and pH *ca.* 10. When an A sol was required with low $SiO_2 : Na_2O$ ratio, the sol as prepared above was made up to the required value with sodium hydroxide solution. Type B sols were made by percolation of a 2·5% (w/w) sodium

silicate solution through a column of cation-exchange resin, Nalcite, until the pH of the bulk effluent had risen to about 7. On concentration to 15% by evaporation at 100°, the pH rose to a final value of about 10. The product with 15% of silica was a slightly turbid liquid, with a $SiO_2 : Na_2O$ ratio about 70. If a B sol was required with a higher $SiO_2 : Na_2O$ ratio the 15% sol was passed through the cation-exchange resin to remove all the sodium, and was then made up to the required $SiO_2 : Na_2O$ ratio with sodium hydroxide solution. Type C sols were prepared by percolating a 2% sodium silicate solution through the cation-exchange resin, to remove all the sodium, and making it up to the required $SiO_2 : Na_2O$ ratio with sodium hydroxide solution. The sol was then evaporated to a 4% silica content. These sols were only very slightly turbid.

(b) *Particle-size Measurement.*—The average molecular weights of the particles in the A and the B sols were determined by Debye's light-scattering technique. The molecular weights found were $250 \times 10^6$ for 15% A sols, and $3 \times 10^6$ for 15% B sols as prepared above. The C sols were of much smaller molecular weight, and this would be a function of the $SiO_2 : Na_2O$ ratio. It would probably be of the order of $10^4$—$10^3$.

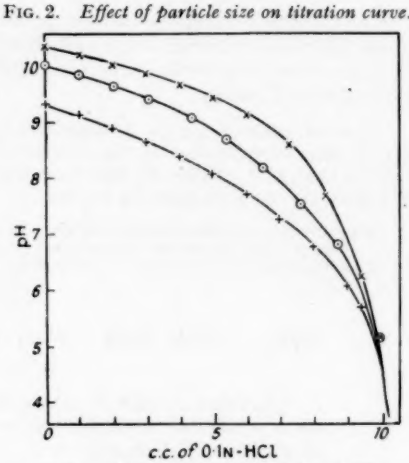
(c) *Titration Technique.*—Since the sols, as prepared, were alkaline, they were generally titrated with acid, instead of removing the sodium and titrating with alkali, but both methods should yield the same result.

FIG. 1. *Effect of electrolyte on titration curve.*




100 *C.c. of* 0·8% *sol titrated with* 0·05N-HCl.
×, No added electrolyte.
⊙, [NaCl] = 0·2N.
+, [NaCl] = 0·4N.

FIG. 2. *Effect of particle size on titration curve.*




50 *C.c. of* 4% *sol titrated with* 0·1N-HCl.
×, A sol. ⊙, B sol. +, C sol.

*Results.*—1. *Effect of electrolyte concentration on titration curves.* The effect of electrolyte (sodium chloride) concentration on the titration curve of an A sol is shown in Fig. 1. The inflection point corresponds to the equivalence of sodium and mineral acid anions. Within experimental error the titration curves of hydrochloric, nitric, and sulphuric acids were identical, indicating that anion adsorption does not play any significant part, which is consistent with the negative charge on the particles. The change in pH caused by a given electrolyte concentration falls off as the $SiO_2 : Na_2O$ ratio of the sol decreases, and becomes negligible at a ratio of about 10.

At relatively high electrolyte concentrations the clear C sols and translucent B sols became cloudy, and it was apparent that their average particle size had increased. This is believed to be a normal preliminary to the gelation of silica sols at high pH.

2. *Effect of particle size on titration curve.* Arising from the observation that the particle size increased during the addition of electrolyte and acid, it was of importance to observe whether the titration curves for sols of the same silica content and $SiO_2 : Na_2O$ ratio depended on the initial particle size. The average particle sizes quoted refer to the initial sols from which those used for the titration were obtained by dilution and adjustment of the $SiO_2 : Na_2O$ ratio. The particle-size distribution in the initial and the final sol need not be the same, but since the qualitative difference in appearance between A, B, and C sols was retained in the final sols, the average particle sizes almost certainly lie in the same order.

It was observed that, the larger the particle size, the higher the pH at a given $SiO_2 : Na_2O$ ratio, electrolyte content, and silica concentration (Fig. 2). Again, the differences between the curves for sols derived from the A, B, and C types were least marked at low $SiO_2 : Na_2O$ ratios, again being insignificant at a $SiO_2 : Na_2O$ ratio of 10.

3. *Effect of* $SiO_2 : Na_2O$ *ratio on particle size.* In sodium silicates of $SiO_2 : Na_2O$ ratio greater than 1 : 1, the silicate ions consist of condensed silicic acid residues, the size and charge depending on the pH. This was indicated by Harman's work (*J. Phys. Chem.*, 1927, **31**, 616) on sodium silicates of varying $SiO_2 : Na_2O$ ratios, and confirmed by that of Naumann and Debye (*J. Phys. Colloid Chem.*, 1951, **55**, 1), who studied the molecular weight of sodium silicates of varying $SiO_2 : Na_2O$ ratio by the light-scattering method. Harman showed that this aggregation was reversible by preparing $Na_2SiO_3$ from sodium silicates of higher $SiO_2 : Na_2O$ ratio and conversely. The breakdown of large particles is a rate process which takes time, and it has been shown here in two ways.

First, the change in conductivity with time was observed on addition of a known amount of a silica sol to a sodium hydroxide solution at pH *ca.* 12. For equal volumes of two sols of the same silica content, the initial rate of change of conductivity varied inversely with the initial particle size.

Secondly, a 15% A sol was made up to various $SiO_2 : Na_2O$ ratios, at the same silica content, and the particle size measured after several weeks. The results are shown in the following table, from which it follows that, while the particle size of an A sol varied with the $Na_2O : SiO_2$ ratio, the rate of change may be quite slow and the particle size will be sensibly constant over a period of minutes or even hours.

| $SiO_2 : Na_2O$ ratio | 250 | 80 | 50 |
|---|---|---|---|
| Average $M$ | $250 \times 10^6$ | $220 \times 10^6$ | $150 \times 10^6$ |

## Discussion

The physical structure of the particle is regarded as similar to that of a sponge, in which the total surface area is much greater than that of a sphere of the same diameter. Such a structure could be heavily hydrated and would also enclose much water, which would lead to the low particle density indicated by light-scattering measurements (see below). It would seem therefore that the equations derived for the titrations of polymeric acids, *e.g.*, by Katchalsky and Gillis (*Rec. Trav. chim.*, 1949, **68**, 879) and Overbeek and Arnold (*ibid.*, 1950, **69**, 192) would be applicable in this case. Both these derivations lead to an equation of the same form, namely,

$$\mathrm{pH} = \mathrm{p}K_a + n \log \alpha/(1 - \alpha)$$

where $\alpha$ is the degree of dissociation of the acid and $n$ a constant. A similar expression has been derived and applied successfully to soluble proteins by Cannan, Palmer, and Kibrick (*Ann. N.Y. Acad. Sci.*, 1941, **41**, 243). To calculate $\alpha$ one needs to know the total number of acid groups present. In the case of the polymers of unsaturated carboxylic acids this is known from the amount of monomer taken, and for proteins it is derived from the length of the "steps" on the titration curves. However, comparison of the curves obtained from polymethacrylic acid (Katchalsky and Spitnik, *J. Polymer Sci.*, 1947, **2**, 432) with Fig. 1 reveals a qualitative difference, since on addition of alkali to acidified silica sols $\Delta\mathrm{pH}/\Delta(\mathrm{alkali})$ decreases steadily, with no indication of an inflection, even for A sols titrated to a point where the $Na_2O : SiO_2$ ratio is greater than 3. $K_a$ being taken to be about $10^{-9}$, as earlier work indicates (see Treadwell, *Trans. Faraday Soc.*, 1935, **31**, 297), the pH at equivalence will be about 11. In the case of a polymeric acid of the same $K_a$ the pH will be higher still. The explanation of the absence of an increase in $\Delta\mathrm{pH}/\Delta(\mathrm{alkali})$ as the equivalence point is approached is believed to lie in the sensitivity of silica particles to alkali at high pH, which causes a lowering of the average particle size and an increase in the total number of ionised and un-ionised ⋮Si·OH groups, both of which will result in the pH being lower than that which would be observed in their absence. As the pH increases, so the rate of dissolution rises and the equivalence point is never reached. The impossibility of determining the total number of acid groups present in the sol thus prevents the use of the above-mentioned theories.

An alternative approach to the problem regards the particle as behaving as if surrounded by a membrane permeable to all ions except the ionised particles. The Donnan theory,

however, cannot be applied because the net charge on the particle is known to vary with the pH (Hazel, *J. Phys. Chem.*, 1938, **42**, 409). As would be expected, the equation derived on the basis that the particle is uncharged does not represent the experimental data. Further, differences due to particle size could not be accounted for on the Donnan theory.

It is, however, possible to explain the results qualitatively by a consideration of the electrical potential of the particle. As for any weak acid partly neutralised, to a first approximation, $[Na^+] = [{:}SiO^-]$, *i.e.*, the sum of the negative charges on all the particles due to ${:}SiO^-$ groups numerically equals the total charge on all the sodium ions. The sodium concentration in a sol as prepared here is referred to as the initial titratable sodium $T_0$, since it can be determined by titration with hydrochloric acid. On addition of this acid to a sol the above equation must be modified to $T_0 = [{:}SiO^-] + [Cl^-]$; and hence $T_0 - [Cl^-] = [{:}SiO^-]$, the left-hand side of this being referred to as the residual titratable sodium, $T_R$. Thus at any point during the titration of an alkaline sol with hydrochloric acid, or during the addition of sodium hydroxide to a pure sol, the total charge, due to silicate ions, on all the particles can be calculated. Hence, if the particle radius is known, the electrical potential can be estimated. The particle radius, $r$, is given by

$$r^3 = \frac{M_s(d_s - 1)}{(d - 1)} \times \frac{3k}{4\pi}$$

where $M_s$ is the molecular weight of the silica skeleton, $k$ is the mass of the hydrogen atom, and $d_s$ and $d$ are the density of silica and of the hydrated particles respectively. Taking $d$ as 1·5 (obtained from light-scattering measurements) and $d_s = 2{\cdot}4$, we have

$$r = M_s^{1/3} \times 7{\cdot}8 \times 10^{-9} \text{ cm.}$$

The charge per particle for a 15% sol is given by

$$q = T_R \times M_s \times 1{\cdot}09 \times 10^{-21} \text{ coulombs}$$

The particle potential is therefore

$$V = T_R \times M_s^{2/3} \times 1{\cdot}6 \times 10^{-3} \text{ volts}$$

$T_R$ being assumed to be 0·02N, $V$ varies from about 70 mv at $M_s = 10^5$ to about 30 v at $M_s = 10^9$. Such high potentials would attract cations (*i.e.*, $H^+$ and $Na^+$) until the net potential of the particle was of the order of 40 mv. This is in agreement with the results of Kargin and Rabinovitch (*Trans. Faraday Soc.*, 1935, **31**, 284), who found that the adsorption of barium ions increased as the pH rose, *i.e.*, as $T_0$ increased.

If it is asumed that the net potential to which the particle is reduced does not vary much with the electrolyte content, then the effect of adding electrolytes will be to alter the relative proportions of adsorbed sodium and hydrogen ions. Thus, on addition of a sodium salt, hydrogen ions will be displaced from the particle into the inter-particle liquid, and so the pH of the solution will fall, as observed. It was noted by Kargin and Rabinovitch (*loc. cit.*) that the number of $H^+$ ions displaced is about one-hundredth of the amount of $Ba^{++}$ ions adsorbed.

The effect of particle size can also be deduced from the above theory, if it is assumed that the adsorption reduces the potential to a value $V_0$ (a few $kT$) which is sensibly constant.

The charge, $q_a$, which has to be adsorbed, per particle, to accomplish this is given by

$$q_a = (T_0 \times M_s^{2/3} \times 1{\cdot}6 \times 10^{-3} - V_0) \times 80r$$

and the charge adsorbed per c.c., $Q_a$, is given by

$$Q_a = (T_0 M_s^{2/3} \times 1{\cdot}6 \times 10^{-3} - V_0) \times 80r \times N$$

where $N$ is the number of particles per c.c.

Now $r = \text{constant} \times N^{-1/3}$ and so

$$Q_a = GT_0 - HN^{2/3}$$

where $G$ and $H$ are constants for a given silica content.

In these circumstances $N \propto M_s^{-1}$, and so as $M$ increases, $Q$ increases, *i.e.*, more adsorption takes place. Hence the pH rises, as observed (Fig. 2).

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# 577. *Silica Sols. Part II.* Conditions of Stability.*

By S. Baxter and K. C. Bryant.

The effect of electrolyte concentration and titratable sodium on the stability of small-particle silica sols has been determined. At low electrolyte concentrations a minimum in the stability–$T_R$ curve was found, but at higher concentrations the stability increased progressively with decrease in $T_R$. As the initial titratable sodium, $T_0$, of a silica sol increases from zero, the stability passes through a maximum and then through a minimum. A qualitative explanation of these observations is given in terms of Verwey and Overbeek's theory.

In earlier work (see Hurd, *Chem. Reviews*, 1938, **22**, 403) the behaviour of silica sols has been related to their pH and silica content, with neglect of electrolyte concentration and particle size. The results already presented (Part I *) show that the pH of a sol is affected by particle size and electrolyte concentration, and in the work described here, the initial and residual titratable sodium are used to describe the silica sols investigated. The extension of this work to include the influence of particle size was prevented by circumstances, though an effect has been observed. The experimental results, together with those of other workers, can readily be explained qualitatively on a theory based on current concepts of colloid stability.

## Experimental

*Sols.*—Unless otherwise stated all the sols used were of the type C described in Part I.

*Gelation Measurements.*—A known amount of dilute hydrochloric acid was added to the sol in a boiling-tube to bring the pH or, more strictly, the value of $T_R$ (see Part I), to the required value. Sodium chloride solution was added until the concentration in the sol was as required (allowance being made for sodium chloride formed from the acid), and the corked tube was placed, after being shaken, in a thermostat at 30°. Gelation was assumed to have taken place when the sol no longer flowed on tilting the tube, the time being measured from the moment of mixing. (The term stability is used synonymously with gelation time in the description and discussion below.)

This method probably lacks the precision of Hurd's " tilted rod " technique (*J. Phys. Chem.*, 1932, **36**, 2194), and the silica content in a given series of measurements varies by $\pm 3\%$ (*i.e.*, $3{\cdot}8 \pm 0{\cdot}1\%$) over extreme values of $T_R$. However, the errors introduced were found to be negligible, and the results reproducible to $\pm 5\%$.

*Results.*—The results given in Part I show that the pH of a silica sol is a function of both the particle size and the electrolyte content, and also that it may vary with time. It was therefore considered desirable to replace the pH scale by that of the titratable sodium content, which is independent of particle size and electrolyte content. Fig. 1 shows stability–$T_R$ curves for various electrolyte concentrations, and it is seen that at low electrolyte concentrations there is a minimum in the curve whereas at the higher electrolyte concentrations the stability rises continuously as $T_R$ decreases. These effects were observed for A, B, and C type sols.

The behaviour of a series of C sols of 0·4N-sodium chloride and different $T_0$ is shown in Fig. 2. The stability passes through a maximum at low values of $T_0$ and a minimum at higher values. The shape of this curve is of fundamental importance for an understanding of the factors affecting the stability of silica sols. It was found that, as $T_0$ was decreased, so $T_R$ at the minimum of stability, expressed as a fraction of $T_0$, increased.

## Discussion

A. *Mechanism of Gelation of Silica Sols.*—It has already been pointed out by Hurd, Pomatti, Spittle, and Alois (*J. Amer. Chem. Soc.*, 1944, **66**, 388) that sols formed above pH 8 are soft, thixotropic, and opaque, while below pH 8 they are hard, elastic, and only slightly turbid. It has been found here that A-type sols always give soft gels while B sols give hard gels below about pH 8.

It therefore appears that there are two mechanisms of gelation, operative at different

* Part I, preceding paper.

pH's. The differences between the two types of gel, and the fact that they are related to sols of different particle size, are explicable on this basis, one mechanism involving the chemical reaction of ⁝Si·OH groups to form Si–O–Si linkages, the other being a physical coagulation. The chemical mechanism is considered first; this will be favoured by high concentrations of ⁝Si·OH groups (*i.e.*, high silica content, high $T_0$, and low $T_R$), high collision number (small particle size), and a low activation energy (absence of steric hindrance). Since chemical bonds join the particles, the gel may be expected to be hard, elastic, and irreversible. Thus C type sols may be expected to form hard gels at low pH's, while it is reasonable that A sols give soft gels at all pH's owing to the low value of the collision number and the steric hindrance in the case of large particles.

The second mechanism is physical and is explained on Verwey and Overbeek's theory of colloidal stability ("Theory of the Stability of Hydrophobic Colloids," Elsevier, 1948). This theory predicts that under certain conditions colloidal particles will be attracted to one another, leading to coalescence and precipitation.

The distance of approach of the centres of two colloidal silica particles is limited by their size and steric factors, but if they can approach close enough they will experience a weak

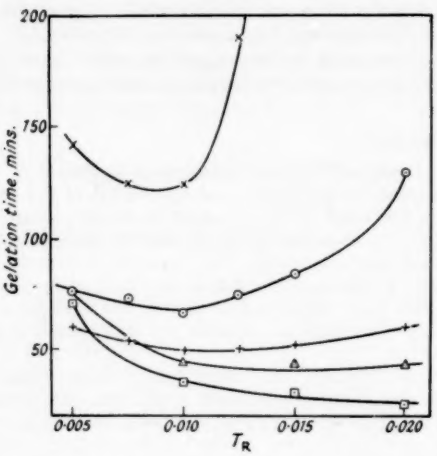


FIG. 1. *Effect of electrolyte concentration on shape of $T_R$–stability curve.*

*Normality of* NaCl:
×, 0·2; ○, 0·3; +, 0·4; △, 0·5; □, 0·6.

attraction. This, repeated throughout the volume of the sol, will produce in time a "brushwood heap" type of structure with contact between particles. This will be a rate process with an activation energy, but this is reduced by increasing the electrolyte concentration, and hence the velocity will be increased. Furthermore, if the separation of the particles requires only a small energy expenditure, then the linkages will readily be broken by agitation; *i.e.*, the gel will be thixotropic.

It can be shown that as the potential of the particles increases the repulsive force similarly increases, leading to a higher potential barrier to coalescence, and so to increased stability. However, as the potential increases, adsorption of oppositely charged ions will take place, and a limiting potential will be reached. It has already been shown (Part I) that sol particles will adsorb cations to reduce their surface potential, which will increase the ionic strength both within and around the highly porous particle. The concentration of cations within the particle is thus higher than that which would exist in the double layer round a solid particle of the same size and of the same potential as that to which the silica particle is finally brought. Hence the concentration gradient at a given distance from the surface in the liquid round the silica particle is greater than at the same point in the double layer round the solid particle. For a given net surface potential and overall ionic strength, therefore, the greater the adsorption the more rapidly will the potential decay in the vicinity of the particles. The variation with interparticle distance of the repulsive forces between charged particles depends, among other things, on the rate of

decay of potential with distance from each particle. Increasing this rate of decay leads to a more rapid change of repulsive force with distance for a given surface potential, and hence to a lower stability.

These two mechanisms are in no way mutually exclusive. The physical mechanism will always be operative, but under suitable conditions it will be reinforced by the chemical combinations.

B. *Experimental Results.*—In view of Debye and Naumann's results (*J. Phys. Colloid Chem.*, 1951, **55**, 1), together with results given in Part I, there seems no doubt that the average particle size of a silica sol at equilibrium is a function of the $SiO_2 : Na_2O$ ratio, the molecular weight falling as $T_0$ increases. The experimental results are most conveniently discussed in terms of Fig. 2, the curve being considered in three sections, *AB*, *BC*, and *CD*. At this point the use of titratable sodium becomes important as a scale of reference instead of pH, since it influences the cationic adsorption. At *A*, corresponding to a pure silica sol, free from sodium ($T_0 = 0$), the surface potential of a small particle is much less than $\frac{3}{2}kT$ and there is no adsorption of cations. As $T_0$ is increased by the addition of sodium hydroxide, the potential increases, and as it approaches a certain maximum value, $V_m$ at *B*, cationic adsorption commences. This adsorption leads to a decrease of the repulsive

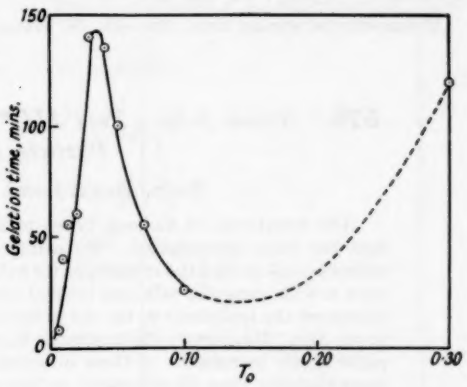


FIG. 2. *Effect of $T_0$ on stability of sols of constant silica content* (3·8%) *and electrolyte content* 0·4N-NaCl.

forces between particles, and hence as $V_m$ is approached there are two opposing effects, namely, the increase of stability due to increasing potential, and the decrease due to the more rapid decay of this potential with distance. At *B*, the second factor takes control, and though $T_0$ increases, the particle potential is kept constant by cationic adsorption, while the stability is progressively lowered by the increased adsorption. As $T_0$ increases further, the particle size is diminishing, and although the potential of the fragments will be approximately the same as before, the total amount of adsorption by the sol will be less than before. This means that the density of adsorbed charges within the particle and its double layer decreases as the particle size decreases. Thus a stabilising effect comes into operation, and as $T_0$ increases further a minimum of stability appears at *C*. After that the colloidal properties of the sol diminish steadily as the particle size falls.

The effect of adding acid to sodium silicate can be explained similarly.

The effect of electrolyte content on the shape of the stability–$T_R$ curve is to be interpreted as due to a decrease in the repulsive force between particles, and hence the formation of larger particles by coalescence, though this need not be followed by gelation until much later. It has been shown (Part I) that on addition of electrolyte to a sol the pH falls, but the titrations were carried out immediately, to avoid gelation. It has been observed repeatedly that the pH of a sol increases on storage, and this is attributed to increase of particle size. The effect of electrolyte in increasing the particle size is shown by the increased opacity immediately upon addition of high electrolyte concentrations, leading to greater cation adsorption, and consequently reduced stability. On addition of hydro-

chloric acid the number of ⋮SiO⁻ groups is reduced, and the cation adsorption decreases. This effect overshadows that due to the increased number of ⋮Si·OH groups and the stability increases. Similarly, if alkali is added to a sol containing sufficient electrolyte, then at some value of $T_0$ gelation will take place immediately.

It has also been observed that as $T_0$ is lowered the value of $T_R$ at the minimum of stability approaches $T_0$. This can also be explained, for the rate of dissolution of silica particles will be very small at low pH, and since the pH is a function of the $SiO_2 : Na_2O$ ratio, the above argument indicates that $T_R$ tends towards $T_0$ as $T_0$ decreases.

It has been stated by several authors that there is a range of pH values between which the stability of silica sols has a minimum value. Values given include 9·5—11 (Freundlich and Cohn, *Kolloid-Z.*, 1926, **39**, 28), 8·3 (Hurd *et al.*, *J. Amer. Chem. Soc.*, 1944, **66**, 388), 6—8 (Ray and Ganguly, *J. Phys. Chem.*, 1930, **34**, 352), 6·5—7·5 (Batchelor, *ibid.*, 1938, **42**, 575), 5·8 (Treadwell, *Trans. Faraday Soc.*, 1935, **31**, 297), and −0·5 to 0·5 (Hurd and Barclay (*J. Phys. Chem.*, 1940, **44**, 847). In the light of the facts and theory presented here, the experimental conditions under which these various values were obtained have been examined in detail. It is considered that there is no real discrepancy between these widely differing pH values, since they refer to different silica concentrations and several methods of varying the stability.

Monsanto Chemicals, Ltd., Ruabon, N. Wales. [*Received, January 18th*, 1952.]

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## 578. *Silica Sols. Part III.* Accelerated Gelation, and Particle Size.*

By S. Baxter and K. C. Bryant

The resistance of various types of silica sol to an accelerated gelation test has been investigated. By using, in most cases, mixtures of sodium chloride and alcohol the conditions for gelation in 1 hour have been determined over a wide range of salt and alcohol concentrations. The addition of acid increased the resistance of the sol to the accelerated gelation test. Also for a given $SiO_2 : Na_2O$ ratio the resistance to accelerated gelation decreased as the particle size increased. If these different particle size sols are brought to the same stability then the titratable sodium is shown to decrease with increase in particle size. The experimental results presented are qualitatively explained on the theory developed in Parts I and II.*

Silica sols of high pH are very stable, and since the viscosity increases continuously but slowly over a period of weeks or months, it is difficult to attach a quantitative value to the gelation time. A direct correlation of stability with such variables as pH, silica content, and electrolyte content would be lacking in precision, and would require an impracticably long time for the accumulation of experimental data. An attempt was therefore made to compare the stabilities of different sols by determining the amount of alcohol required to bring about gelation in a short time. In this connection, an investigation was undertaken into the relation between the electrolyte content of a sol and the amount of alcohol which caused gelation in 1 hour.

### Experimental

*Sols.*—The sols used were all of the type A and B (see Part I, *J.*, 1952, 3017). The silica content was about 15% (w/w), and the $T_0$ values varied from 0·14N to 0·02N.

From methods of preparation, the sols contained small amounts of sodium chloride and sodium sulphate, respectively, which had to be included in the total electrolyte content. These were found conductometrically or potentiometrically to be about 0·001—0·01N.

*Gelation Technique.*—The normal practice in gelation measurements is to fix the conditions and to measure the gelation time. In this instance, however, it was more convenient to reverse this procedure and to determine the conditions of electrolyte content and dielectric constant

* Part II, preceding paper.

which produced gelation in a fixed time of 1 hour. Unless the silica content of the gelling mixture was being deliberately varied, 10 ml. of 15% sol were always used, being added rapidly to 10 ml. of the mixed electrolyte solution, the non-electrolyte, and water. Gelation was assumed to have taken place when the sol no longer flowed on inversion of the tube after 1 hour at 30°. The results obtained were consistent and reproducible to 0·1 ml. of non-electrolyte.

It was observed that, if an excess of alcohol was added, no sharp onset of gelation was observed, though the viscosity increased immediately, followed later by syneresis.

Unless otherwise specified the subsequent results refer to experiments carried out with sodium chloride as the electrolyte and ethyl alcohol as the non-electrolyte.

*Results.*—1. *Effect of alcohol.* Since, in any theoretical approach to the stability of colloids, the dielectric constant of the medium will play an important rôle, the non-electrolyte concentration was represented by the dielectric constant of the medium which, for different non-electrolyte concentrations, was calculated from Akerlof's data (*J. Amer. Chem. Soc.*, 1932, **54**, 4125), with neglect of the water chemically bound to the silica particles and any absorbed non-electrolyte. The dielectric constants were measured at a frequency of 2 Mc/sec., so these approximate to the static values.

On using sodium chloride and alcohol it was found that a plot of log (electrolyte concentration) against the dielectric constant of the medium for sols which gelled in 1 hour gave a straight line

FIG. 1. *Log (electrolyte content)–dielectric constant plots for typical A sols.*

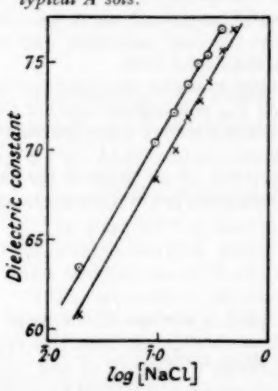
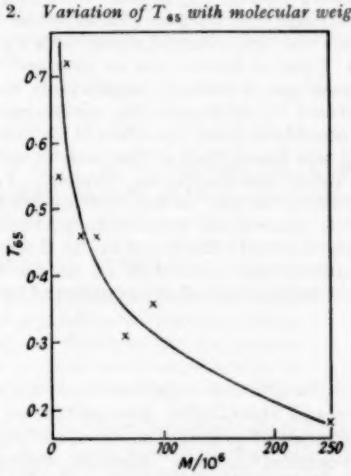



FIG. 2. *Variation of $T_{65}$ with molecular weight.*




over a wide range of electrolyte content (Fig. 1). Both A and B sols became white and opaque during gelation, and the gels obtained were thixotropic. Different sols gave lines which were parallel within the experimental error, but were by no means coincident. It was soon established that this was not due to minor variations in silica content, and the position of the line was defined arbitrarily by the value of the dielectric constant corresponding to 0·1N-sodium chloride, which will be referred to as the alcohol constant.

It was later found that with other non-electrolytes such as higher alcohols and dioxan a linear relationship was not obtained, and the dielectric constant corresponding to a given electrolyte content varied with the nature of the non-electrolyte.

2. *Effect of variation of electrolyte.* It was found that with a series of the potassium salts of various strong acids, such as the chloride, bromide, nitrate, sulphate, ferricyanide, and ferrocyanide, the behaviour was almost independent of the nature and valency of the anion.

With various chlorides it was found that the gelling efficacy varied with the valency of the cation, and slightly within a given valency. Thus at the same non-electrolyte concentration bivalent ions such as calcium and barium produced gelation in 1 hour at a normality only about one-quarter of that required by a univalent electrolyte. This ratio was much less than that found by Freundlich and Cohn (*Kolloid Z.*, 1926, **39**, 28) though here the conditions were not strictly comparable.

This sensitivity to cations, and insensitivity to anions, is in agreement with the negative charge on the particles, shown by electrophoretic measurements.

3. *Effect of addition of mineral acids.* It was found, however, that on lowering the pH by adding dilute hydrochloric, nitric, or sulphuric acid to the test mixtures more alcohol was required for a given electrolyte concentration, *i.e.*, the stability to the 1-hour gelation test had increased. The slope of the log (electrolyte content)–D.C. plot was unaltered, but the line was displaced to a lower alcohol constant. The displacement was independent of the acid, for equivalent additions.

It has already been shown that the pH of a silica sol varies with the electrolyte content (Part I, *loc. cit.*), and it was also found that the pH was raised by the addition of alcohol. It therefore follows that it is possible to prepare a series of sols of different electrolyte and alcohol content with the same stability but different pH. This is another example of the weakness inherent in the use of pH in discussions of silica sols. As before, this difficulty can be surmounted by the use of the residual titratable sodium, $T_R$, for while the pH varies in these sols, they will all have the same $T_R$ if made from the same initial sol.

Since the alcohol constant of a sol can be lowered to almost any required value by the addition of acid, a series of sols of different initial alcohol constants can be brought to the same level of stability by the addition of a suitable amount of acid. In this work the stability selected was that corresponding to an alcohol constant of 65, and the value of $T_R$ at that level is designated $T_{65}$.

This property, $T_{65}$, appears to be independent of the value of $T_0$ for sols of the same particle size, though this could only be shown for those sols with large particles and low $T_0$, in which the particle size only changed slowly with $T_0$.

4. *Effect of particle size on stability.* When a B sol was heated in an autoclave at a high temperature, it became progressively more turbid, and finally resembled an A sol. It was confirmed by light-scattering measurements that the particle size had increased, and thus it was possible to study the effect of particle size on stability in the 1-hour test.

It was found that as the particle size increased the alcohol constant increased, indicating that the sol was less stable. Similarly, $T_{65}$ had decreased, and $T_{65}$ was plotted against the particle size of the sol. It was therefore possible to obtain a semi-quantitative value for the average particle size without recourse to light-scattering measurements.

The scatter in the points in Fig. 2 is probably due to a variation of the shape of the particle-size distribution curve with $T_0$, for the molecular weights measured are weight-averages, and do not imply identical distributions of particle size.

## Discussion

In the gelation experiments described here the gel formed is always of the same type, white and thixotropic, irrespective of the pH. It is thus considered that the physical mechanism of gelation, as developed by Verwey and Overbeek (" Theory of the Stability of Lyophobic Colloids," Elsevier, Amsterdam, 1948), is predominant at all pH's considered, for at high values the concentration of ⁝Si·OH groups will be low, while even at low values the collision number will be low and the energy barrier to condensation probably high for steric reasons. Verwey and Overbeek's theory indicates that a lowering of the dielectric constant will cause a decrease in stability, but does not explain the linear log (electrolyte content)–D.C. relation found with alcohol and sodium chloride. However, in addition to lowering the dielectric constant, specific absorption effects with different non-electrolytes will doubtless affect the stability of the sols.

The increase in turbidity on mixing the components of the gelation mixture indicates that the particle size is increasing, leading to increased cationic adsorption. As with sols containing a high concentration of electrolyte, the addition of acid leads to neutralisation of the ⁝SiO⁻ groups, with a consequent reduction in cationic adsorption and hence increase of stability. By analogy with gelation experiments at high and low electrolyte concentrations, it might have been expected that at low salt contents the stability would decrease as the pH fell, but this is offset by the effect of the alcohol. On the present theory, the effect of lowering the pH is to reduce the concentration of ⁝SiO⁻ groups, and hence the amount of adsorption of cations decreases. This leads to a less rapid decay of potential with distance and so, a constant particle-potential being assumed, to increased stability. It would be expected that the stability in the 1-hour gelation test would pass through a maximum and eventually decrease, but in the present series of experiments it was not possible to explore

this region owing to limitations on the amount of acid and alcohol possible in the experimental scheme used.

The increase of particle size when a B sol was autoclaved is to be accounted for as the progressive attainment of an equilibrium. The rate at which condensation takes place between the ⋮Si·OH groups of different particles may be virtually zero at room temperature, or even at 100°, but if the activation energy is high these condensations may take place much more rapidly at higher temperatures. (If the temperature is too high, then gelation takes place, which is probably to be understood as a result of the rapid condensation to form a loose network of particles throughout the system instead of a comparatively slow growth to form large particles.) This equilibrium is approached from the opposite direction in the production of A sols by the dispersion of an elastic gel by autoclaving it with sodium hydroxide. As the particle size increases at a constant $T_0$ value, then (Part I) the charge concentration of adsorbed ions within the particle increases also. A constant particle potential being again assumed, then the fall off of potential with distance will become more rapid as the particle size increases, giving rise to less stable conditions, *i.e.*, the alcohol constant increases with particle size at a constant $T_0$. Clearly, then, the $T_{65}$ value will decrease as particle size increases, a result shown experimentally in Fig. 2.

The authors acknowledge their indebtedness to Mr. H. A. Vodden for particle-size measurements and to Mr. H. O. Williams for assistance with the experimental work, and to Professor Sir Eric K. Rideal, F.R.S., for helpful discussions throughout.

MONSANTO CHEMICALS LTD., RUABON, N. WALES. [*Received, January 18th*, 1952.]

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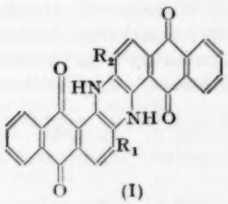
## 579. *Chemistry of Indanthrone. Part VII.* The Action of Piperidine on 3 : 3′-Dihalogenoindanthrones and the N-Methyl Derivatives of 3 : 3′-Dibromoindanthrone.*

By WILLIAM BRADLEY and HARRY E. NURSTEN.

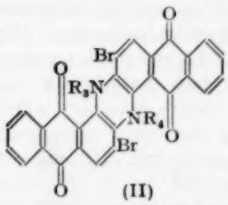
In Part VI * it was shown that heating 3 : 3′-dihalogeno- and 3 : 3′-dialkylsulphonyl derivatives of indanthrone with piperidine or quinoline caused the replacement of the substituents by hydrogen. Study of the mechanism of the replacement has now indicated that the reaction involves reduction of a quinone nucleus.

IN Part VI * it was shown that indanthrone (I; $R_1 = R_2 = H$) resulted when 3 : 3′-diethylsulphonylindanthrone (I; $R_1 = R_2 = SO_2Et$) was heated with dry piperidine or quinoline, and in the present investigation an attempt has been made to elucidate the mechanism of the reaction.

The course of the reaction between amines and 3 : 3′-disubstituted indanthrones depends on circumstances. Heating with dry piperidine has no effect on 3 : 3′-dibromoindanthrone (I; $R_1 = R_2 = Br$) (Part VI), but when the reaction is carried out in naphthalene at a higher temperature indanthrone is formed. 3 : 3′-Dichloroindanthrone



(I)

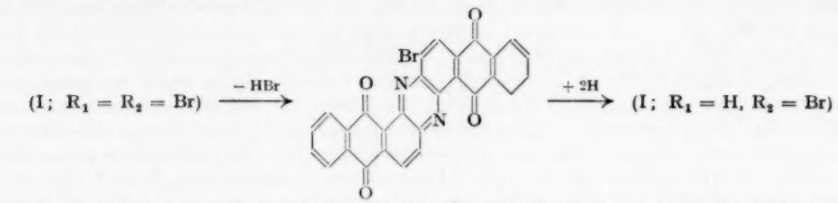


(II)

(I; $R_1 = R_2 = Cl$) behaves differently; the product in this case is 3-chloro-3′-piperidinoindanthrone (I; $R_1 = Cl$, $R_2 = NC_5H_{10}$). There is no reaction with either of the dihalogenoindanthrones when morpholine is used instead of piperidine. The replacement

* Part VI, *J.*, 1951, 2177.

of chlorine by piperidine is expected, but the replacement of bromine by hydrogen involves reduction. A possible mechanism is reduction of the carbonyl groups followed by the elimination of hydrogen bromide (Part VI). Alternatively, elimination of halogen could occur by the following means :




In order to decide between the alternatives, the reaction of piperidine with the *N*-methyl and *NN'*-dimethyl derivatives of 3 : 3'-dibromoindanthrone has been studied. The *N*-methyl derivative (II; $R_3$ = Me, $R_4$ = H) afforded *N*-methylindanthrone; the *NN'*-dimethyl compound (II; $R_3 = R_4$ = Me) yielded 3 : 3'-dibromo-*N*-methylindanthrone (II; $R_3$ = Me, $R_4$ = H) and *NN'*-dimethylindanthrone. The elimination of a methyl group is not unexpected because of the experiments reported below, but the elimination of bromine from 3 : 3'-dibromo-*NN'*-dimethylindanthrone indicates the occurrence of reduction and supports the view of the reaction put forward in Part VI. It could be suggested that the first step in the reaction is the replacement of a halogen substituent by the piperidino-group, and that the product then loses piperidine forming the azine of the dehalogenated compound. This view is unlikely to be correct because 3-chloro-3'-piperidinoindanthrone is not an unstable compound, nor does the proposed mechanism explain the formation of *NN'*-dimethylindanthrone from its 3 : 3'-dibromo-derivative.

N-*Methyl Derivatives of* 3 : 3'-*Dibromoindanthrone.*—The methylation of 3 : 3'-dibromoindanthrone by methyl toluene-*p*-sulphonate and potassium carbonate in tetrachlorobenzene yields the *N*-methyl derivative (II; $R_3$ = Me, $R_4$ = H) or the *NN'*-dimethyl derivative (II; $R_3 = R_4$ = Me) according to the reaction conditions. At the same time there is formed a trimethyl derivative the structure of which is not yet established. The constitution of the *NN'*-dimethyl derivative has been confirmed by its synthesis from 1 : 3-dibromo-2-methylaminoanthraquinone, sodium acetate, and copper acetate in *o*-dichlorobenzene.

The *NN'*-dimethyl derivative is relatively unstable; on exposure to light a solution in α-chloronaphthalene gives the *N*-methyl derivative. The *N*-methyl compound also results when the *NN'*-dimethyl derivative is heated with concentrated sulphuric acid at 100° for 30 minutes. These two reactions establish the constitution of the *N*-methyl derivative. The *NN'*-dimethyl compound shows the property of thermochromism in organic solvents : solutions are blue when cold but become red-violet when heated. There are also unexpected features in the absorption spectra of this compound.

In concentrated sulphuric acid indanthrone and its *N*-methyl, *NN'*-dimethyl, and 3 : 3'-dibromo-derivatives show almost identical spectra. In the same solvent the *N*-methyl and *NN'*-dimethyl derivatives of 3 : 3'-dibromoindanthrone resemble one another but differ from indanthrone. With respect to indanthrone their absorption occurs at shorter wave-lengths and the intensity is smaller, particularly in the case of the *NN'*-dimethyl compound (see Table). In α-chloronaphthalene or chlorobenzene the main

| Compound | Absorption max. in conc. sulphuric acid | | | | | |
|---|---|---|---|---|---|---|
| | $m\mu$ | $\varepsilon \times 10^{-4}$ | $m\mu$ | $\varepsilon \times 10^{-4}$ | $m\mu$ | $\varepsilon \times 10^{-4}$ |
| Indanthrone ..... | 470 | 1·25 | 570 ** | 0·41 | >720 | >1·19 |
| *Indanthrone derivative.* | | | | | | |
| * *N*-Methyl ..... | 470 | 1·07 | 570 ** | 0·41 | 720 | 1·15 |
| * *NN'*-Dimethyl ..... | 472 | 1·02 | 580 ** | 0·33 | >720 | >1·26 |
| 3 : 3'-Dibromo ..... | 470 | 1·32 | 580 | 0·42 | >730 | >1·92 |
| 3 : 3'-Dibromo-*N*-methyl ..... | <440 | >1·69 | | | 575 | 1·23 |
| 3 : 3'-Dibromo-*NN'*-dimethyl ... | <460 | >0·54 | | | 575 | 0·31 |

* Part I (*J.*, 1951, 2129). ** Inflexion.

absorption maxima of all the following 3 : 3′-disubstituted indanthrones, diethylsulphonyl-, dibutylsulphonyl-, dichloro-, dibromo-, and di-*tert.*-butyl-, occur in the range 705—725 mμ (*J.*, 1951, 2177, 2179). In *o*-dichlorobenzene the main absorption maxima of *N*-methyl- and *NN′*-dimethyl-indanthrones occur at 665 and 655 mμ, whereas in α-chloronaphthalene the maximum absorption of the 3 : 3′-dibromo-derivatives of these two compounds has been displaced to 610 mμ ($\varepsilon = 1{\cdot}50 \times 10^4$) and 580 mμ ($\varepsilon = 1{\cdot}21 \times 10^4$). The unusual features of the absorption spectra of the *N*-methyl and the *NN′*-dimethyl derivative of 3 : 3′-dibromoindanthrone are typical of non-planar compounds (cf. Braude *et al.*, *J.*, 1949, 1890; Jeffreys and Knott, *J.*, 1951, 1028; Kiprianov and Ushenko, *Bull. Acad. Sci. U.R.S.S., Classe Sci. chim.*, 1950, 492). Similar compounds are likewise non-planar (Remington, *J. Amer. Chem. Soc.*, 1945, **67**, 1838; Brown, Widiger, and Letang, *ibid.*, 1939, **61**, 2597; von Braun, *Ber.*, 1916, **49**, 1101).

Thermochromism due to the formation of diradicals has been reported in the case of dianthronyl (Grubb and Kistiakowsky, *J. Amer. Chem. Soc.*, 1950, **72**, 419; Theilacker, Kortüm, and Friedheim, *Chem. Ber.*, 1950, **83**, 508), and thermochromism due to zwitterion formation has been discussed for 2-*spiro*bis-2-[naphtho(1′ : 2′-5 : 6)pyran] by Schönberg, Mustafa, and Askar (*J. Amer. Chem. Soc.*, 1951, **73**, 2876), Koelsch (*J. Org. Chem.*, 1951, **16**, 1362), and Knott (*J.*, 1951, 3038), but neither of these phenomena would provide a satisfactory explanation of the thermochromism of 3 : 3′-dibromo-*NN′*-dimethylindanthrone. Thus with indanthrone derivatives the wave-length of maximum absorption is not greatly influenced by the dielectric constant of the medium. *NN′*-Dimethylindanthrone exhibits an absorption maximum at 645 mμ ($\varepsilon = 2{\cdot}00 \times 10^4$) in toluene (dielectric constant = 2·4), and at 655 mμ ($\varepsilon = 2{\cdot}51 \times 10^4$) in nitrobenzene (dielectric constant = 36).

It remains a possibility that the thermochromism of 3 : 3′-dibromo-*NN′*-dimethylindanthrone is the result of interchange between *cis*- and *trans*-forms.

The two procedures for methylating 3 : 3′-dibromoindanthrone described in the experimental portion afford, respectively, (*a*) the *NN′*-dimethyl derivative (26%) and a trimethyl derivative (18%), and (*b*) the *N*-methyl derivative (6%) and the same trimethyl derivative (7·5%). The formation of the trimethyl derivative does not involve methylation of either nitrogen or carbon, because the compound readily yields 3 : 3′-dibromoindanthrone when it is heated with sulphuric acid; under the same conditions 3 : 3′-dibromo-*N*-methylindanthrone is recovered unchanged. In the presence of Raney nickel the trimethyl derivative absorbs only 0·41 mol. of hydrogen. Under the same conditions 3 : 3′-dibromo-*NN′*-dimethylindanthrone takes up 1·72 mols. of hydrogen. The trimethyl compound appears to be an *O*-derivative, but further work is needed to establish its constitution.

In preparing 1 : 3-dibromo-2-methylaminoanthraquinone a number of practical difficulties were encountered. Bromine (1·1 mols.) in acetic acid reacted with 2-methylaminoanthraquinone to form 3-bromo-2-methylaminoanthraquinone in 40% yield. Increasing the proportion of bromine to 2·5 mols. gave a similar result, 29% of the methylaminoanthraquinone being recovered. *N*-Bromosuccinimide (2 mols.) in benzene gave 1-bromo-2-methylaminoanthraquinone (54%) together with a small amount of the 3-bromo-compound. In one experiment 1 : 3-dibromo-2-methylaminoanthraquinone (21%) and the 1-bromo-compound (26%) were obtained. The 1 : 3-dibromo-derivative did not result when the 1-bromo-compound was treated with bromine in acetic acid or when the 3-bromo-compound was treated with *N*-bromosuccinimide. Bromine (2 mols.) in nitrobenzene gave a mixture of the 1- and the 3-bromo-derivative, the proportions varying with the temperature of the reaction. In attempting to methylate 2-amino-1 : 3-dibromoanthraquinone we found that the use of methyl toluene-*p*-sulphonate and potassium carbonate in *o*-dichlorobenzene (yield 31%) or polychlorobenzenes (b. p. *ca.* 230°) (yield 19%) was successful. The product was 1 : 3-dibromo-2-methylaminoanthraquinone identical with the compound referred to above.

When heated in chlorobenzene with sodium acetate and a small proportion of copper acetate, 1-bromo-2-methylaminoanthraquinone gave *NN′*-dimethylindanthrone (5%) together with 2-methylaminoanthraquinone and flavanthrone. *NN′*-Dimethylindanthrone

was prepared similarly from 2-bromo-1-methylaminoanthraquinone; the result is interesting because when nitrobenzene is employed as the solvent *NN'*-dimethylindanthrone is not obtained (Part II, *J.*, 1951, 2148). Further, the formation of flavanthrone in a condensation of this kind is unusual, the most nearly related instance being the formation of 3 : 3'-di-*tert.*-butylflavanthrone in the self-condensation of 2-amino-1-bromo-3-*tert.*-butylanthraquinone (Part V, *J.*, 1951, 2176; cf. also D.R.-P. 172,733). 2-Amino-1-chloroanthraquinone has been stated to yield flavanthrone (Kopetschni and Wiesler, *Sitzungsber. Akad. Wiss. Wien*, 1922, **131**, II, *B*, 41), but the result has been disputed (Maki and Mine, *J. Soc. Chem. Ind. Japan*, 1944, **47**, 522B).

Under similar conditions 1 : 3-dibromo-2-methylaminoanthraquinone gave 1 : 3-dibromo-*NN'*-dimethylindanthrone (39%), identical with the compound already described.

## Experimental

M. p.s below 360° are corrected.

*Action of Piperidine on* 3 : 3'-*Dibromoindanthrone.*—3 : 3'-Dibromoindanthrone (Found : Br, 26·7. Calc. for $C_{28}H_{12}O_4N_2Br_2$ : Br, 26·7%) (0·1 g.) was refluxed for 24 hours with piperidine (5 c.c.) and naphthalene (20 g.). The resulting suspension was cooled, mixed with benzene, and then filtered. The residue, after being washed with hot benzene, consisted of red-violet crystals (60 mg.) (Found : N, 5·9; Br, 2·3%) having a coppery lustre. The main portion of the product was indanthrone.

Under similar conditions 3 : 3'-dibromoindanthrone was recovered unaltered after being heated with a mixture of morpholine and naphthalene. The bromo-compound was much less soluble in this reagent.

*Action of Piperidine on* 3 : 3'-*Dichloroindanthrone.*—Under the conditions used with the dibromo-analogue 3 : 3'-dichloroindanthrone (Found : Cl, 14·1. Calc. for $C_{28}H_{12}O_4N_2Cl_2$ : Cl, 13·9%) (0·1 g.) afforded lustrous violet-blue needles (0·09 g.) (Found : N, 7·2; Cl, 6·1. $C_{33}H_{22}O_4N_3Cl$ requires N, 7·5; Cl, 6·3%). 3-*Chloro*-3'-*piperidinoindanthrone* was only slightly soluble in pyridine; it dissolved with a blue colour in alkaline sodium dithionite (hydrosulphite).

Under the same conditions, 83% of the dichloro-compound was recovered when a mixture of morpholine and naphthalene was used.

3 : 3'-*Dibromo*-NN'-*dimethylindanthrone.*—(*a*) Finely divided 3 : 3'-dibromoindanthrone (0·5 g.) was heated in an open flask with anhydrous potassium carbonate (2·3 g.) and mixed polychlorobenzenes (50 c.c.; b. p. 230—250°) until moisture had been expelled. A solution of methyl toluene-*p*-sulphonate (3·1 g.) in polychlorobenzene (50 c.c.) was then added gradually during 30 minutes to the boiling suspension. Thirty minutes after the addition was complete, the boiling solution was filtered and the filtrate concentrated to small volume. Chromatography on alumina, with chlorobenzene to develop the bands, gave two main zones. The more mobile was dark blue, the less mobile red-violet. The column was cut and the two colouring matters were eluted with hot chlorobenzene. The extracts were concentrated, then cooled and mixed with acetone.

(i) The dark blue band gave lustrous crystals of 3 : 3'-*dibromo*-NN'-*dimethylindanthrone* (Found : C, 57·6; H, 2·5; N, 4·5; Br, 25·7; NMe, 9·7. $C_{30}H_{16}O_4N_2Br_2$ requires C, 57·3; H, 2·5; N, 4·5; Br, 25·5; NMe, 9·2%) (Found, under the conditions of methoxyl determination : NMe, 4·6%). The compound (yield, 0·138 g.) had m. p. 376° (decomp.). It was paramagnetic. There was no colour change when methyl-alcoholic potassium hydroxide was added to its blue solution in cold pyridine; in the same test 3 : 3'-dibromoindanthrone changed from blue to green. The solution in concentrated sulphuric acid was yellow-brown, becoming yellow on the addition of a small volume of water; a violet-blue precipitate formed on further dilution. Addition of nitric acid to the solution in sulphuric acid gave a deep red-brown colour. The compound dissolved in alkaline sodium dithionite, forming an orange solution. It was only very sparingly soluble in acetone or ligroin. The blue solution in organic solvents became red-violet when heated; the colour change was reversed on cooling. Hydrogenation in α-chloronaphthalene acetic-acid in the presence of Raney nickel caused the absorption of 1·72 mols. of hydrogen.

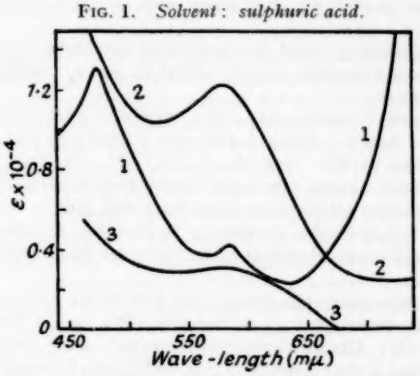
(ii) The red-violet band afforded minute, violet needles (0·097 g.) of a trimethyl derivative of 3 : 3'-dibromoindanthrone (A) (see below).

(*b*) 1 : 3-Dibromo-2-methylaminoanthraquinone (0·1 g.) was heated under reflux for 5·5 hours in *o*-dichlorobenzene (7 c.c.) containing finely powdered anhydrous sodium acetate

(0·1 g.) and a very small quantity of cupric acetate. The suspension changed in colour from red to blue. It was filtered hot, and the filtrate was cooled and chromatographed on alumina. Several zones formed and from these were isolated (i) unchanged 1 : 3-dibromo-2-methylaminoanthraquinone (0·028 g., 28%), (ii) 3 : 3′-dibromo-*NN*′-dimethylindanthrone (0·031 g., 39%), and (iii) unidentified product (0·018 g.). Fraction (ii) was identified by its absorption spectrum and colour reactions. When the heating was carried out for 24 hours the yields were (ii) 42%, and 2-bromo-3-methylaminoanthraquinone 17%. Phenolic compounds were formed under both conditions, the amount increasing with duration of heating; there was no flavanthrone.

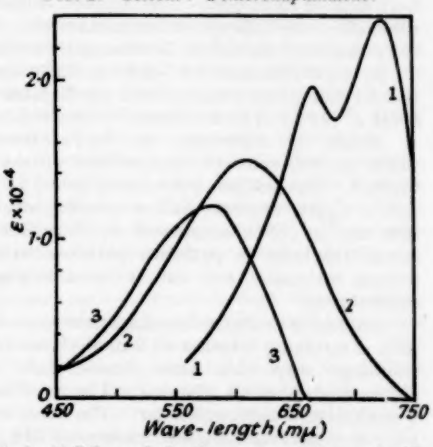
*Action of Piperidine on* 3 : 3′-*Dibromo*-NN′-*dimethylindanthrone*.—The dimethyl compound (0·039 g.) was refluxed in piperidine (10 c.c.) for 4 hours. The resulting solution was cooled and then mixed with acetone. The minute, lustrous crystals (0·011 g.) which separated were chromatographed on alumina; several green and blue zones were formed. The colouring matter was extracted from the main blue zone by chlorobenzene containing a small volume of pyridine. The extract was concentrated, and then mixed with ligroin. Blue needles (0·004 g.) having a

FIG. 1. *Solvent*: *sulphuric acid.*




1, 3 : 3′-*Dibromoindanthrone*: *maxima*: λ >730, ε >19,200; λ 580, ε 4200; λ 470, ε 13,200.
2, 3 : 3′-*Dibromo*-N-*methylindanthrone*: *maxima*: λ 575, ε 12,300; λ <440, ε >16,900.
3, 3 : 3′-*Dibromo*-NN′-*dimethylindanthrone*: *maxima*: λ 575, ε 3100; λ <460, ε >5400.

FIG. 2. *Solvent*: *α-chloronaphthalene.*




1, 3 : 3′-*Dibromoindanthrone*: *maxima*: λ 722, ε 23,800; λ 665, ε 19,700.
2, 3 : 3′-*Dibromo*-N-*methylindanthrone*: *maximum*: λ 610, ε 15,000.
3, 3 : 3′-*Dibromo*-NN′-*dimethylindanthrone*: *maximum*: λ 580, ε 12,100.

coppery lustre separated. These did not contain halogen; they were identified as *NN*′-dimethylindanthrone by their absorption spectrum in *o*-dichlorobenzene, by the colour of their solution in pyridine, concentrated sulphuric acid, and alkaline sodium dithionite, and by the absence of a colour change on the addition of methyl-alcoholic potassium hydroxide to the blue solution in pyridine.

When the reaction was carried out for 2 hours, chromatography showed the presence of 3 : 3′-dibromo-*N*-methylindanthrone (see below).

3 : 3′-*Dibromo*-N-*methylindanthrone*.—(*a*) Finely divided 3 : 3′-dibromoindanthrone (0·5 g.) was methylated under the conditions used for the dimethyl derivative except that four times the volume of polychlorobenzenes was employed, the methylating agent was added in one portion and the duration of heating was 2 hours. The resulting suspension was filtered hot from inorganic material and unchanged dibromoindanthrone, and the violet-blue filtrate was evaporated to a small volume and then chromatographed on alumina. Development with chlorobenzene gave two main zones. (i) The more mobile band was blue; the colouring matter was extracted with chlorobenzene, and the extract concentrated and then cooled. Blue crystals (0·029 g.) having a coppery lustre separated. These consisted of the *monomethyl* derivative, m. p. 356° (decomp.) (Found: C, 57·3; H, 2·4; N, 4·3; Br, 25·7; NMe, 4·7. $C_{29}H_{14}O_4N_2Br_2$ requires C, 56·7; H, 2·3; N, 4·6; Br, 26·1; NMe, 4·8%). It dissolved somewhat easily in organic solvents with a blue colour; the solution in acetic anhydride became violet when heated, but the blue colour

returned on cooling. The solution in pyridine remained blue after the addition of methyl-alcoholic potassium hydroxide. It dissolved in alkaline sodium dithionite with a red-brown colour, and in concentrated sulphuric acid forming a brown solution; this became yellow on the addition of water and a blue precipitate separated on further dilution. (ii) The less mobile band was red-violet. The colouring matter, a *trimethyl* derivative of 3 : 3′-dibromoindanthrone, was obtained, on elution, in the form of minute, violet needles, which charred above 300° (Found : C, 58·1; H, 2·6; N, 4·7; Br, 24·15; NMe, 13·6. $C_{31}H_{18}O_4N_2Br_2$ requires C, 57·9; H, 2·8; N, 4·4; Br, 24·9; NMe, 13·6%). It was identical with the product (A) obtained in the preparation of 3 : 3′-dibromo-*NN*′-dimethylindanthrone.

3 : 3′-Dibromotrimethylindanthrone dissolved in concentrated sulphuric acid with an olive colour; a red-violet precipitate formed on addition of the solution to water. When the temperature was allowed to rise during the dilution the precipitate was blue. The red-violet solution in pyridine became browner on the addition of methyl-alcoholic potassium hydroxide. The compound dissolved in alkaline sodium dithionite with a green colour. Hydrogenation in α-chloronaphthalene–acetic acid in the presence of Raney nickel caused the absorption of 0·41 mol. of hydrogen. The reduced compound was green; when kept for a long time in an atmosphere of hydrogen it became brown. Heated at 100° for 30 minutes with sulphuric acid, the compound yielded 3 : 3′-dibromoindanthrone.

(*b*) 3 : 3′-Dibromo-*NN*′-dimethylindanthrone (0·050 g.) and concentrated sulphuric acid (3 c.c.), heated on a steam-bath for 30 minutes, gave a solution which, added to water, yielded 0·046 g. (94%) of 3 : 3′-dibromo-*N*-methylindanthrone.

*Action of Piperidine on* 3 : 3′-*Dibromo*-N-*methylindanthrone.*—The dibromo-compound (0·044 g.) was refluxed in piperidine (10 c.c.) for 7 hours. The solution was cooled and then filtered. The halogen-free residue (0·005 g.) (Found : N, 6·5. Calc. for $C_{29}H_{16}O_4N_2$ : N, 6·1%) was a violet, microcrystalline powder which showed almost the same absorption maximum (660 mμ) in *o*-dichlorobenzene as *N*-methylindanthrone (absorption maximum 665 mμ). The colour reactions in pyridine, pyridine with methyl-alcoholic potassium hydroxide, alkaline sodium dithionite, and concentrated sulphuric acid were identical with those of *N*-methylindanthrone.

1-*Bromo*-2-*methylaminoanthraquinone.*—*N*-Bromosuccinimide (0·6 g.) was heated for 3 hours with a refluxing solution of 2-methylaminoanthraquinone (0·4 g.) in benzene (200 c.c.). The resulting suspension was filtered hot, and the filtrate was concentrated and then chromatographed on alumina. The most mobile band was faint pink; it contained 3-bromo-2-methylaminoanthraquinone. The main zone was orange-red. Extraction with acetone gave 1-*bromo*-2-*methylaminoanthraquinone* (0·29 g.), and this crystallised from acetic acid in yellowish-orange needles, m. p. 198—199° (decomp.) (Found : C, 57·5; H, 3·3; N, 4·8; Br, 25·6. $C_{15}H_{10}O_2NBr$ requires C, 57·0; H, 3·2; N, 4·4; Br, 25·3%). It dissolved in concentrated sulphuric acid with a greenish-yellow colour and in alkaline sodium dithionite forming a red solution. The orange solution in pyridine became yellow-green on the addition of a drop of methyl-alcoholic potassium hydroxide. In one preparation the main orange-red band was preceded by a yellow band from which were isolated yellow needles of 1 : 3-*dibromo*-2-*methylaminoanthraquinone* (21%), m. p. 189—190° (Found : C, 45·5; H, 2·3; N, 3·7; Br, 40·65. $C_{15}H_9O_2NBr_2$ requires C, 45·6; H, 2·3; N, 3·5; Br, 40·5%). These showed the same reactions with concentrated sulphuric acid and sodium dithionite as the 1-bromo-compound. The solution in pyridine was yellow, changed to green by the addition of methyl-alcoholic potassium hydroxide.

3-*Bromo*-2-*methylaminoanthraquinone.*—Bromine (1 c.c.) was dissolved in acetic acid (100 c.c.). A portion of the solution (12·7 c.c.) was added to 2-methylaminoanthraquinone (0·5 g.) in hot acetic acid (70 c.c.). After 2 hours at 100° the solution was diluted and cooled. 3-*Bromo*-2-*methylaminoanthraquinone* (0·27 g.; 40%) separated. Repeated crystallisation from acetic acid gave orange needles, m. p. 241° (Found : C, 56·6; H, 3·1; N, 4·25; Br, 25·7. $C_{15}H_{10}O_2NBr$ requires C, 57·0; H, 3·2; N, 4·4; Br, 25·3%). The compound resembled the 1-bromo-isomer in its reactions with concentrated sulphuric acid and alkaline sodium dithionite. The orange solution in pyridine became yellow green on the addition of methyl-alcoholic potassium hydroxide.

In the early stages of the bromination pink crystals, m. p. 200—213° (decomp.), frequently separated. In contact with alcohol they readily formed 3-bromo-2-methylaminoanthraquinone.

Bromination of 2-methylaminoanthraquinone in nitrobenzene yielded a mixture of the 1- and the 3-bromo-derivative. To a solution of 2-methylaminoanthraquinone (0·2 g.) in nitrobenzene (20 c.c.) were added 11·6 c.c. of a solution prepared from bromine (1 c.c.) and

nitrobenzene (100 c.c.). After 2 hours at 100° the solvent was distilled in steam and the residual orange powder (0·3 g.; m. p. 175—185°) was chromatographed on alumina from benzene. Two orange bands formed. From the more mobile was obtained 3-bromo-2-methylaminoanthraquinone (0·12 g., 45%), and from the other 1-bromo-2-methylaminoanthraquinone (0·073 g., 27%). Bromination at 140—150° for 4 hours gave 23% of the 3-bromo- and 8% of the 1-bromo-derivative.

When 2·7 c.c. of a solution of bromine (1 c.c.) in acetic acid (100 c.c.) were added to 1-bromo-2-methylaminoanthraquinone (0·1 g.) in acetic acid (20 c.c.) and heated at 100° for 2·5 hours the product was a mixture. Chromatography on alumina gave several zones; the only compound identified was 2-methylaminoanthraquinone (13%).

The starting material was recovered in 40% yield when 3-bromo-2-methylaminoanthraquinone (0·2 g.) was heated under reflux with *N*-bromosuccinimide (0·12 g.) in benzene (50 c.c.).

1 : 3-*Dibromo-2-methylaminoanthraquinone*.—A suspension of potassium carbonate (2 g.) in a solution of 2-amino-1 : 3-dibromoanthraquinone (1·4 g.) in mixed polychlorobenzenes (100 c.c.; b. p. *ca.* 230°) was heated to boiling to expel moisture, and then methyl toluene-*p*-sulphonate (2 g.) was added and the refluxing continued for 2 hours. The product was filtered hot, and the filtrate was concentrated to small volume and then chromatographed on alumina. Development with benzene gave several bands. The most mobile, orange band eluted with acetone afforded 1 : 3-dibromo-2-methylaminoanthraquinone (0·27 g. 19%), identical with one of the products obtained by heating 2-methylaminoanthraquinone with *N*-bromosuccinimide. A similar preparation carried out in *o*-dichlorobenzene gave the dibromo-compound in 31% yield, but when chlorobenzene was used 87% of the starting material was recovered unchanged.

There was no indication of methylation having occurred when 2-amino-1 : 3-dibromoanthraquinone was treated as follows: (*a*) stirred with 20% oleum and 40% formaldehyde at 0° or 100° (cf. U.S.P. 2,091,235); (*b*) treated with paraformaldehyde and sulphuric acid at 0° or 100°; or (*c*) heated with methyl sulphate and sulphuric acid for 2 hours at 100°. There was no indication of acylation when 2-amino-1 : 3-dibromoanthraquinone was refluxed for 30 minutes with toluene-*p*-sulphonyl chloride and pyridine.

*Self-condensation of 2-Bromo-1-methylaminoanthraquinone*.—2-Bromo-1-methylaminoanthraquinone (0·1 g.), finely powdered anhydrous sodium acetate (0·1 g.), and a very small proportion of cupric acetate were heated under reflux for 24 hours in *o*-dichlorobenzene (7 c.c.). The solution changed in colour from red to violet-blue. It was filtered hot and the filtrate chromatographed on alumina. Development with *o*-dichlorobenzene gave several zones. Amongst the less mobile was a blue band and from this the colouring matter was eluted by means of quinoline at 150°. The eluate, concentrated and then mixed with acetone, gave *NN'*-dimethylindanthrone in very small amount, identified by its colour reactions and absorption spectrum in *o*-dichlorobenzene.

*Self-condensation of 1-Bromo-2-methylaminoanthraquinone*.—Under the same conditions 1-bromo-2-methylaminoanthraquinone gave 0·004 g. of *NN'*-dimethylindanthrone. In addition a more mobile, red band on the alumina column was found to contain 2-methylaminoanthraquinone. Further flavanthrone was found in the residue obtained at the first filtration stage.

Under the same conditions 3-bromo-2-methylaminoanthraquinone gave a complex product containing 46% of unchanged initial material.

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## 580. *Chemical Actions of Ionising Radiations in Solutions. Part X.* The Action of X-Rays on Ammonia in Aqueous Solution.*

By Tyson Rigg, George Scholes, and Joseph Weiss.

It has been found that ammonia in dilute aqueous solutions is oxidised to nitrite at room temperature by the action of the radicals produced by irradiation with *X*-rays, provided that molecular oxygen is present. The influence of pH, concentration, and other factors on this process has been investigated. Hydroxylamine is apparently not involved as an intermediate. Attention has been drawn to the related biological process.

Ionising radiations can deaminate various amino-compounds in aqueous solution, leading to the formation of ammonia. These compounds include amino-acids (Stein and Weiss, *J.*, 1949, 3256; Dale, Davies, and Gilbert, *Biochem. J.*, 1949, **45**, 93), and nucleic acids (Scholes, Stein, and Weiss, *Nature*, 1949, **164**, 709; Scholes and Weiss, *ibid.*, 1950, **166**, 640). The amino-group can also be liberated to a smaller extent in the form of hydroxylamine, the yield of which depends on the nature of the substrate (Scholes and Weiss, *ibid.*, 1951, **167**, 693).

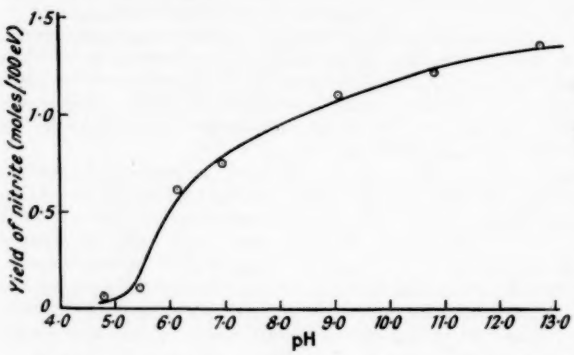



Fig. 1.

pH-*Dependence of the formation of nitrite from* 0·1M-$(NH_4)_2SO_4$ *solutions, in the presence of air (constant dose of* $1·6 \times 10^4$ *rep).*

On the other hand, irradiation of ammonia solutions with *X*-rays in the presence of air results in an oxidation to nitrite but in this case no hydroxylamine is formed. The yield of nitrite was found to depend markedly on the pH (Fig. 1) and it can be shown that it is essentially dependent on the concentration of the free ammonia, according to the equilibrium, $NH_3 + H^+ \rightleftharpoons NH_4^+$. This is confirmed by the results presented in Fig. 2, showing that the production of nitrite in a dilute solution of ammonium sulphate ($5 \times 10^{-4}$M) at pH 9·62 follows practically the same course as in a more concentrated solution ($10^{-1}$M) at pH 6·90, in which the concentration of the free $NH_3$ molecules is approximately the same.

There was no difference in the behaviour of air- or oxygen-saturated solutions (Fig. 3, curve *a*). In experiments with evacuated solutions, however, no oxidation could be detected—the solutions were tested for nitrite, hydroxylamine, and hydrazine. From these observations it is concluded that the presence of oxygen is essential in the radiation-induced oxidation of ammonia. The ionic yield for the formation of nitrite is rather low, and the curve for air shown in Fig. 3 exhibits no break which would correspond to exhaustion of atmospheric oxygen (Stein and Weiss, *J.*, 1949, 3245; Rigg, Stein, and Weiss, *Proc. Roy. Soc.*, 1952, *A*, **211**, 375). The similarity between the results with air and oxygen can be explained on the grounds that here, even in air-saturated solutions, sufficient oxygen is always available under the conditions of the experiments represented by Fig. 3.

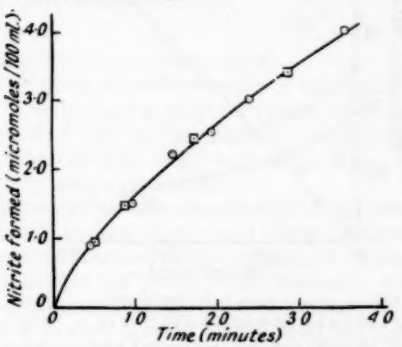
The yield–dose plots for the formation of nitrite display a marked curvature. This

* Part IX, *J.*, 1951, 1247.

is presumably a consequence of further oxidation of the nitrite since addition of the latter before irradiation results in a much lower rate of nitrite production, as shown by the initial slopes of curves *b* and *a* in Fig. 3. This view is further supported by the results, presented in Fig. 4, of experiments in more concentrated ammonia solutions, in which the initial steep slope is maintained up to a greater total dose. From this it appears that ammonia ($NH_3$) in sufficiently high concentrations can exert a "protective" effect on the nitrite. Further oxidation of the nitrite presumably leads to formation of nitrate (cf. Fricke and Hart, *J. Chem. Phys.*, 1935, **3**, 365).

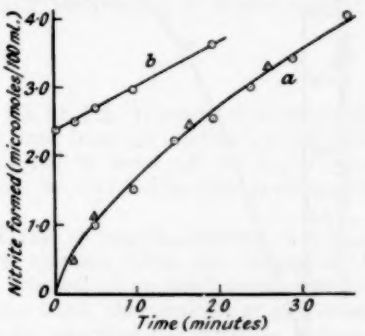
The absence of detectable amounts of hydroxylamine in any of these experiments indicates that, if this substance is an intermediate in the oxidation of ammonia, it must be present in a stationary concentration of the order of *less* than $10^{-6}$ mole/l. and should therefore be itself oxidised very rapidly; for instance, in the oxidation of a $10^{-1}$M-solution of ammonia any hydroxylamine, acting as an intermediate, would, therefore, have to be oxidised about $10^6$ times faster than the ammonia.

FIG. 2. *Dependence of the formation of nitrite on the time of irradiation, in the presence of air (dose rate* 3200 *rep/min.).*




□, $5 \times 10^{-4}$M-$(NH_4)_2SO_4$ *at* pH 9·6.
⊙, 0·1M-$(NH_4)_2SO_4$ *at* pH 6·9.

FIG. 3. *Dependence of nitrite formation on the time of irradiation (dose rate* 3200 *rep/min.).*




*a*, 0·1M-$(NH_4)_2SO_4$ *at* pH 6·9.
⊙ *In the presence of air.*
△ *In the presence of oxygen* (1 *atm.*).
*b*, 0·1M-$(NH_4)_2SO_4$ *at* pH 6·9 *in air with added nitrite (initial concn.* $2{\cdot}4 \times 10^{-3}$M).

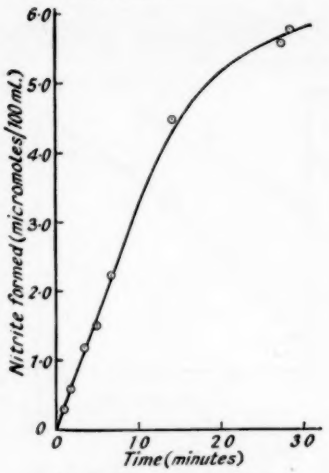
To investigate this point ammonia solutions were irradiated in the presence of hydroxylamine. Some difficulty was at first encountered in these experiments owing to some loss of hydroxylamine, but this was overcome by simultaneous analysis of blank and irradiated solutions. The results obtained (Fig. 5) show that the hydroxylamine is oxidised only about $5 \times 10^3$ times faster than ammonia under similar conditions. This can be deduced, *e.g.*, from curve *b'* (Fig. 5) which shows that hydroxylamine (initial concentration of about $1 \times 10^{-5}$ mole/l.) after 5 minutes of irradiation decreases by $0{\cdot}55 \times 10^{-6}$ mole/100 ml., whereas the nitrite formed from the ammonia (at a concentration of about $10^{-1}$ mole/l.), during the same time interval, is of the order of $1 \times 10^{-6}$ mole/100 ml. Therefore, it may be concluded that hydroxylamine is not formed here as an intermediate in the oxidation process. Although some of the added hydroxylamine is oxidised there is no effect on the overall nitrite yield (Fig. 5, curve *a*), which suggests that, under these conditions, ammonia and hydroxylamine are oxidised independently to nitrite in about the same yield.

*Discussion.*—It is well known that ammonia in the gaseous phase in the presence of oxygen is relatively easily converted into oxides of nitrogen by means of a suitable catalyst. However, in aqueous systems, ammonia is relatively inert towards ordinary oxidising agents (cf. Mellor, "Treatise on Inorganic and Theoretical Chemistry," Vol. VIII, p. 205). In biological systems under aërobic conditions on the other hand, it is easily oxidised to nitrous acid. It is, therefore, of interest that ammonia in aqueous systems can be likewise

oxidised to nitrite, at room temperature, in the presence of oxygen, if the reaction is initiated by the free radicals produced by the irradiation of the aqueous solution with *X*-rays.

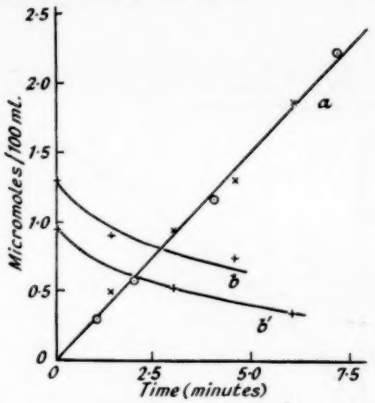
One of the most interesting features of the oxidation under the influence of *X*-rays is the important rôle of oxygen, without which no reaction appears to take place. Recently, other evidence has indicated that the presence of oxygen during irradiation may lead to marked changes in the nature of the end-products. For example, in the presence of oxygen, pyruvic acid is formed on irradiation of alanine solutions (Johnson, Scholes, and Weiss, *Science*, 1951, **114**, 112) and *o*-benzoquinone is obtained from phenol solutions under suitable conditions (Stein and Weiss, *J.*, 1951, 3265). It is known that the primary

FIG. 4. *Dependence of the formation of nitrite on the time of irradiation, in the presence of air (dose rate 3200 rep/min.).*




. 0·1M-$(NH_4)_2SO_4$ *at* pH 9·0.

FIG. 5. *Dependence of the formation of nitrite on the time of irradiation in the presence and absence of added hydroxylamine, in air.*




a, ⊙ *Nitrite formed from* 0·1M-$(NH_4)_2SO_4$ *at* pH 9.
× *As above but with added* $NH_2$·OH ($1 \times 10^{-5}$M).
b *and* b′ *show the decrease of the added hydroxylamine:*
b *for initial concn. of* $1{\cdot}3 \times 10^{-5}$M-$NH_2$·OH.
b′ ,, ,, $0{\cdot}95 \times 10^{-5}$M-$NH_2$·OH.

*net* process in the irradiation of water by ionising radiations (Weiss, *Nature*, 1944, **153**, 748; *Trans. Faraday Soc.*, 1947, **43**, 314) is represented by

$$H_2O \longrightarrow H + OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

which may be followed in the presence of molecular oxygen by the very fast reaction:

$$H + O_2 \longrightarrow HO_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

One function of the oxygen, therefore, may be to inhibit the recombination of the radicals produced according to reaction (1) and it may, on the other hand, also react with some other intermediate radicals produced in the system. In general, the presence of molecular oxygen should enhance oxidative processes and in certain systems the participation of oxygen may be essential. The oxidation of ammonia falls into the latter category.

The nature of the intermediate steps in the oxidation of ammonia is, as yet, a matter of some conjecture, but it follows from the experimental results discussed above that hydroxylamine is very unlikely to be an intermediate.

Neither hydrazine nor hydroxylamine could be detected in the experiments carried out *in vacuo*. However, under the conditions prevailing in these experiments recombination of the radicals primarily formed according to reaction (1), or back-reactions such as

$NH_2 + H \longrightarrow NH_3$, may account for the absence of any observable oxidation. Such back-reactions may be inhibited by oxygen and a plausible mechanism, involving $NH_2$ radicals, may be represented as follows :

$$NH_3 + OH \longrightarrow NH_2 + H_2O \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$NH_2 + O_2 \longrightarrow NH_2O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4a)$$

where it is assumed that reaction (*4a*) is very fast and is followed by a dehydrogenation reaction, *e.g.* :

$$NH_2O_2 + OH \longrightarrow HNO_2 + H_2O \quad . \quad . \quad . \quad . \quad . \quad (4b)$$

Alternatively, the $NH_2$ radicals formed by reaction (3) could undergo disproportionation, leading to the formation of ammonia and the imine radical (NH), *viz.* :

$$2NH_2 \longrightarrow NH_3 + NH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

It is known that the imine radical is rather unstable and decomposes to ammonia and nitrogen and possibly also hydrogen (cf. Sidgwick, "The Chemical Elements and their Compounds," Oxford Univ. Press, 1950, p. 711). This could also explain the lack of formation of any oxidation products in *vacuo*, whereas in the presence of oxygen one could have the reaction :

$$NH + O_2 \longrightarrow HNO_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

leading directly to nitrous acid.

The yield of nitrite reaches a maximum of $G \sim 1{\cdot}3$ (cf. Fig. 1) where $G$ = moles/100 ev. This leads to a yield per radical of $\gamma \sim 0{\cdot}26$, obtained from the relation $\gamma = WG/100$, where $W$ is the energy required to form one radical pair in water ($W$ for liquid water has been taken to be 19·4 ev, Rigg, Stein, and Weiss, *loc. cit.*). This yield is compatible with the above mechanism.

Some preliminary experiments on the effects of *X*-rays on aqueous solutions of primary aliphatic amines show that these substances are oxidised with the formation of hydroxylamine and small amounts of nitrite. The presence of an alkyl group therefore must modify the course of the reaction. On the other hand, the presence of a hydroxyl group (as in $NH_2{\cdot}OH$) does not apparently change the nature of the products, although hydroxylamine is more readily attacked than ammonia (Fig. 5).

In view of these results it seems likely that certain other oxidations of ammonia may involve radical intermediates. For instance, nitrite is formed when alkaline ammonia solutions are treated with copper in the presence of oxygen (Müller, *Z. Elektrochem.*, 1926, **32**, 109). It is also of interest that although perbenzoic acid reacts with ammonia and aliphatic amines it apparently does not react with their salts (Botvinnik, *J. Gen. Chem. U.S.S.R.*, 1946, **16**, 863).

As is well known, the formation of nitrite from ammonium salts is of great importance in the biochemistry of the nitrogen cycle. Virtanen (*Suomen Kem.*, 1945, **18**, *B*, 48) has presented evidence that hydroxylamine is apparently an intermediate; other authors, however (*e.g.*, Pethica, Roberts, and Winter, *Nature*, 1949, **163**, 408), have disputed this. The biological process has now been simulated by radicals produced by *X*-rays in the presence of oxygen and apparently without the intermediate formation of hydroxylamine.

## Experimental

Solutions of ammonium sulphate (100 ml.) were irradiated with *X*-rays from a Victor Maximar set operating at 200 kv and 15 ma, the experimental arrangement being essentially that employed by Farmer, Stein, and Weiss (*J.*, 1949, 3241). Doses were determined by the ferrous sulphate actinometric method (Miller, *J. Chem. Phys.*, 1951, **18**, 79); the mean dose rate was found to be about 3200 rep/minute.

Triply distilled water was used throughout for the solutions, ordinary distilled water being redistilled from permanganate and then from dilute orthophosphoric acid. Buffer solutions were used to ensure that the pH decrease during irradiation did not exceed 0·2 pH unit. For the range of pH 5—8, Sørensen phosphate buffer was used and, for pH 8—10, borate–sodium

hydroxide mixtures were found to be quite satisfactory; for higher pH values sodium hydroxide alone was added.

Experiments were performed (*a*) in air, (*b*) with solutions saturated with oxygen at 1 atm. (oxygen being bubbled through the solution for about 30 minutes), and (*c*) *in vacuo*. Evacuation of solutions was carried out as follows: the vessel containing the solution to be irradiated was de-aërated as fully as possible by means of a Hyvac oil-pump and (oxygen-free) nitrogen was then admitted to ordinary pressure; this process was repeated once more. After this procedure it was found (on a gas-analysis apparatus) that less than 0·01 ml. of non-condensable gas remained. Alkaline ammonia solutions cannot, however, be subjected to this treatment on account of the volatility of ammonia and, for this reason, unbuffered solutions were used and the necessary amount of sodium hydroxide was placed in a side-arm of the irradiation vessel until evacuation was completed. The hydroxide was then tipped into the ammonium sulphate solution, the pH being measured after irradiation.

Commercial " oxygen-free " nitrogen was further purified by passage through a solution of 10% sodium hydrosulphite (dithionite) in 10% sodium hydroxide solution containing 1% of sodium anthraquinone-2-sulphonate (Fieser, *J. Amer. Chem. Soc.*, 1924, **46**, 2639), through a copper sulphate solution, then through a calcium chloride and soda-lime scrubber, and finally through a tube containing copper gauze at ~380°. The gas so purified was found to contain less than one part of oxygen per million when tested with a trypaflavine–silica gel (Kautsky and Hirsch, *Z. anorg. Chem.*, 1935, **222**, 126; Franck and Pringsheim, *J. Chem. Phys.*, 1943, **11**, 21).

*Determination of Reaction Products.—Nitrite and hydroxylamine.* Endres and Kaufman's colorimetric method (*Annalen*, 1937, **530**, 184) was adopted; when applied in conjunction with a " Spekker " colorimeter it gave accurate results with nitrite or hydroxylamine concentrations down to $1 \times 10^{-6}$M.

For determination of nitrite, 10 ml. of sample (neutral) were treated with 2 ml. of sulphanilic acid solution and 2 ml. of α-naphthylamine reagent, the mixture was set aside for 15 minutes until the colour (red) was fully developed, and the whole finally diluted to 50 ml. The optical density was then measured, a green filter (Ilford 604) being used. The colour was found to be stable for several hours.

Hydroxylamine was determined as nitrite after oxidation with iodine. To 10 ml. of sample (neutral) were added 2 ml. of sulphanilic acid solution and 0·2 ml. of iodine solution (0·65 g. in 100 ml. of glacial acetic acid). After 15 minutes, the excess of iodine was removed with the minimum amount of thiosulphate (2% w/v) and the solution immediately treated with the α-naphthylamine reagent as above.

By a combination of these two methods nitrite and hydroxylamine, even if present together, could be accurately determined.

The sulphanilic acid reagent consisted of a solution of 5·25 g. in 400 ml. of water to which, after the solid had dissolved, 100 ml. of glacial acetic acid were added. The α-naphthylamine reagent was prepared as follows: 3·0 g. of α-naphthylamine were boiled in 500 ml. of water for several minutes, the solution filtered hot, and the filtrate acidified with 25 ml. of glacial acetic acid. The latter reagent is comparatively unstable and was used only if the reagent blank values were very low.

*Hydrazine.* In the vacuum experiments tests for hydrazine were carried out by Pesez and Petit's method (*Bull. Soc. chim.*, 1947, 122), with a *p*-dimethylaminobenzaldehyde reagent. This reagent gives a yellow-red colour with hydrazine and is capable of detecting one micromole per 100 ml. Hydroxylamine does not interfere but nitrite gives a similar colour to hydrazine.

Throughout this investigation " AnalaR " chemicals were used.

One of us (T. R.) thanks the Cumberland Education Committee for a Scholarship, and the Northern Council of the B.E.C.C. for financial support.

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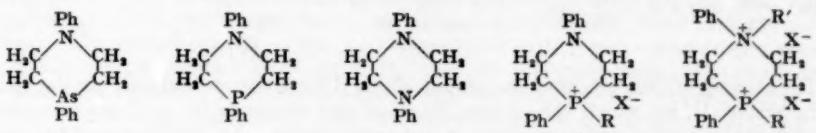
## 581. *The Preparation and Properties of Hexahydro-1 : 4-diphenyl-1 : 4-azaphosphine.*

By Frederick G. Mann and Ian T. Millar.

Phenylphosphinebis(magnesium bromide) reacts with di-(2-bromoethyl)-aniline to form the azaphosphine named in the title. The properties of the azaphosphine have been studied in detail, because it forms a eutropic series with 1 : 4-diphenylpiperazine and hexahydro-1 : 4-diphenyl-1 : 4-azarsine. Although many of the properties of the azaphosphine are similar to those of the azarsine, others are not intermediate between those of the piperazine and those of the azarsine.

It has been shown by Beeby and Mann (*J.*, 1951, 886) that phenylarsinebis(magnesium bromide), $Ph{\cdot}As(MgBr)_2$, reacts with di-(2-bromoethyl)aniline to form hexahydro-1 : 4-diphenyl-1 : 4-azarsine (I), and we have now investigated the analogous reaction with phenylphosphinebis(magnesium bromide), $Ph{\cdot}P(MgBr)_2$. This reagent was first prepared by Job and Dusollier (*Compt. rend.*, 1927, **184**, 54) and later by Lecoq (*Bull. Soc. chim. Belge*, 1933, **42**, 199), utilising the interaction of phenylphosphine, $Ph{\cdot}PH_2$, and ethylmagnesium bromide. We find that the preparation of this reagent, like that of its arsenic analogue (Beeby, Cookson, and Mann, *J.*, 1950, 1920), is greatly improved by the use of phenylmagnesium bromide : this advantage would of course be nullified in those (comparatively rare) cases in which the traces of diphenyl, which accompany the phenylmagnesium bromide, interfere with the ready isolation of the product obtained subsequently by the use of the phenylphosphinebis(magnesium bromide).




(I) (II) (III) (IV) (V)

The compound $Ph{\cdot}P(MgBr)_2$ reacts with di-(2-bromoethyl)aniline to form hexahydro-1 : 4-diphenyl-1 : 4-azaphosphine (II), a colourless crystalline compound of m. p. 89—90°, which undergoes no appreciable oxidation on exposure to dry air at room temperature. It acts as a weak base, forming a dihydrochloride but an unstable monohydriodide and a monopicrate; the tertiary phosphine group permits the ready formation of co-ordination compounds, such as dibromobis(hexahydro-1 : 4-diphenyl-1 : 4-azaphosphine)palladium, $(C_{16}H_{18}NP)_2PdBr_2$.

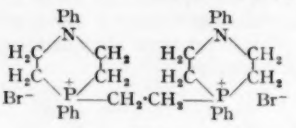
It is of great interest to compare the properties of our new azaphosphine (II) with those of the azarsine (I) and of 1 : 4-diphenylpiperazine (III), since the three compounds form a eutropic series. The piperazine (III) also forms a dihydrochloride but when heated with methyl iodide even at 100° forms only a monomethiodide (Dunlop and Jones, *J.*, 1909, **95**, 419); our own attempts to form diquaternary salts of the piperazine have at present failed. Beeby and Mann (*loc. cit.*) have shown that the azarsine (I) gives only monoquaternary salts, in which quaternisation occurs on the tertiary arsine group; the tertiary amine group in these salts is, however, not entirely inactivated, since it will give salts with strong acids such as hydrobromic acid.

Our investigation shows clearly, however, that in certain respects the azaphosphine (II) does not lie intermediate in properties between the azarsine (I) and the diamine (III). It combines with methyl bromide to form a monomethobromide (IV; R = Me, X = Br) and with methanolic methyl bromide at 100° to form a monomethobromide monohydrobromide (V; R = Me, R′ = H, X = Br); similarly, an excess of ethyl bromide even at 100° yields only a monoethobromide (IV; R = Et, X = Br), and again the reaction if carried out in ethanol gives the monoethobromide monohydrobromide (V; R = Et, R′ = H, X = Br). There is strong evidence, cited below, that when monoquaternisation

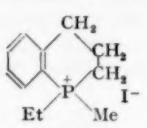
occurs in this way, it is the tertiary phosphine, and not the amine group, which has undergone reaction. In this respect the azaphosphine (II) resembles the azarsine (I).

When, however, the azaphosphine (II) is treated with cold methyl iodide, a vigorous reaction occurs with the formation of the crystalline monomethiodide (IV; R = Me, X = I), but when either the azaphosphine or its monomethiodide is boiled with methyl iodide under reflux, the dimethiodide (V; R = R′ = Me, X = I) is formed; this salt is unstable and when gently heated reverts to the monomethiodide. Similarly the azaphosphine when heated with an excess of methyl toluene-*p*-sulphonate gives a very deliquescent product which is evidently the dimethotoluene-*p*-sulphonate, since when treated with sodium picrate it gives the crystalline dimethopicrate (V; R = R′ = Me, X = $C_6H_2O_7N_3$). It appears therefore that, of the three compounds (I), (II), and (III), only the azaphosphine (II) will form diquaternary salts.

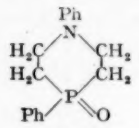
In view of this property of the azaphosphine, and the fact that ethylene dibromide will readily form a cyclic diquaternary salt with a suitable ditertiary arsine or with 1 : 4-dimethylpiperazine (Glauert and Mann, *J.*, 1950, 682; Mann and Mukherjee, *J.*, 1949, 2298), we have attempted to combine the azaphosphine with the dibromide in this way. In spite of the use of a variety of conditions, however, we have isolated only *s*-ethylenebis(hexahydro-1 : 4-diphenyl-1 : 4-azaphosphonium) dibromide (VI), a compound in which the tertiary nitrogen atoms have become markedly inert, for no further quaternisation with methyl iodide could be obtained. The formation of (VI) is, of course, precisely similar to the behaviour of the azarsine (I) under similar conditions.



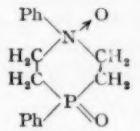
(VI)



(VII)



(VIII)



(IX)

The monoquaternary salts of the azaphosphine (II) are therefore closely similar to those of the arsine (I), for which very strong chemical and spectroscopic evidence was adduced to show that quaternisation occurred on the arsine group. Dr. N. Sheppard has investigated the monomethiodide of the azaphosphine and reports: " It was required to attempt to decide whether the monomethiodide of the azaphosphine had the structure (IV; R = Me, X = I) or whether the methyl group had united with the tertiary amine group to form a quaternary ammonium salt. Chemical evidence indicated that this compound had the former structure, and it was hence decided to compare its spectrum in the region of P–C stretching vibration frequencies with that of an authentic quaternary phosphonium salt having similar groups attached to the phosphorus atom: for this purpose, 1-ethyl-1 : 2 : 3 : 4-tetrahydro-1-methylphosphinolinium iodide (VII) (Beeby and Mann, *J.*, 1951, 411) was chosen. The spectra of both compounds were therefore obtained from 700 to 550 cm.$^{-1}$, a Hilger D209 infra-red spectrometer with a potassium bromide prism being used and the crystalline compounds incorporated as Nujol mulls.

" It is known from infra-red and Raman spectroscopic studies that P–C stretching modes of vibration have frequencies in the range 775—625 cm.$^{-1}$ [*e.g.*, trimethylphosphine, 708 and 653 cm.$^{-1}$ (Rosenbaum, Rubin, and Sandberg, *J. Chem. Phys.*, 1940, **8**, 366); trimethylphosphine oxide, 750 and 671 cm.$^{-1}$ (Daasch and Smith, *ibid.*, 1951, **19**, 22)]. The higher frequencies of this type are liable to be badly overlaid by absorption bands due to $CH_2$ groups and to the aromatic rings present in both the compounds under investigation, but it was considered that there was a reasonable chance that the lower frequency P–C vibration might give comparable absorption bands in the region of 700—600 cm.$^{-1}$ for the two substances, if they were of closely related structure.

" The spectrum of (VII) in the region of 700—550 cm.$^{-1}$ consisted of a single band of medium strength at 640 cm.$^{-1}$, whereas that of the methiodide (IV; R = Me, X = I) consisted of two bands at 640 (medium) and 690 cm.$^{-1}$ (strong). The strong 690-cm.$^{-1}$ absorption band is very probably a frequency originating in the monosubstituted benzene rings of the methiodide (Sheppard, *J. Inst. Petroleum*, 1951, **37**, 95), and it seems reasonable

to assign tentatively the 640-cm.$^{-1}$ band, common to both spectra, as being the corresponding P–C stretching frequencies of the two molecules. Although it would be necessary to investigate the spectra of a much wider range of quaternary phosphorus derivatives before such an assignment could be taken as entirely reliable, it appears probable that the new methiodide is therefore a quaternary phosphonium salt, *i.e.*, that it has the structure (IV; R = Me, X = I)."

The oxidation of the phosphine (II) also gives evidence for the greater reactivity of the phosphine compared with the amine group. When an acetone solution of the phosphine was treated either with a 3% solution of hydrogen peroxide at room temperature for 24 hours, or with a 30% solution at 70—80° for 8 hours, the colourless crystalline monoxide, m. p. 143—144°, was obtained. The evidence that this compound is the phosphine oxide (VIII), and not the amine oxide, is as follows: (*a*) It can also be obtained by the oxidation of the azaphosphine with ethanolic chloramine-T, a reagent which is known to convert tertiary phosphines readily into their oxides but is apparently without action on tertiary amines (Mann and Pope, *J.*, 1922, **121**, 1052; Chaplin and Mann, *J.*, 1937, 527). (*b*) Dr. R. N. Haszeldine, who has investigated the infra-red absorption spectrum of the oxide, finds that it shows a moderately strong band at 7·87 μ, which can be assigned to the P:O linkage. Here again the behaviour of the azaphosphine (II) is analogous to that of the azarsine (I).

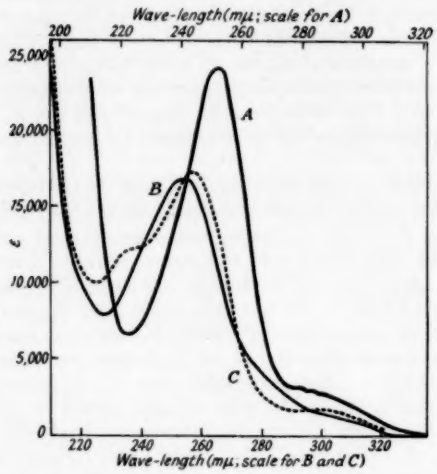
When, however, a solution of the azaphosphine in acetic acid was similarly treated with 30% hydrogen peroxide at 70—80° for 7 hours, stable colourless crystals were ultimately obtained, the composition of which, after they had been recrystallised from ethanol and then dried at 50°/0·5 mm., indicated that the compound was the monohydrate of the dioxide (IX) combined with one molecular equivalent of hydrogen peroxide, *i.e.*, the compound had the composition $C_{16}H_{18}O_2NP,H_2O_2,H_2O$. It is noteworthy that Bennett and Glynn (*J.*, 1950, 211) showed that 1 : 4-diphenylpiperazine (III) when similarly oxidised formed a dihydrated dioxide which also crystallised with 1 mol. of hydrogen peroxide, *i.e.*, it had the composition $C_{16}H_{18}O_2N_2,H_2O_2,2H_2O$. They concluded that the hydrogen peroxide unit formed a hydrogen-bonded bridge between the two oxygen atoms of the dioxide, which, they considered, must therefore have the *cis*-configuration. Our compound, like that of Bennett and Glynn, gave an immediate blue colour when treated in aqueous solution with potassium iodide–starch.

It has been shown by Beeby and Mann (*loc. cit.*) that, when the azarsine (I) is boiled with hydriodic acid, the phenyl group attached to the arsenic atom is replaced by an iodine atom, the heterocyclic ring remaining unaffected; this reaction is shown by several types of heterocyclic tertiary arsines having an aryl group linked to the arsenic atom (Lyon, Mann, and Cookson, *J.*, 1947, 662; Beeby, Cookson, and Mann, *J.*, 1950, 1917; Beeby, Mann, and Turner, *J.*, 1950, 1923). No such fission of the phenyl group occurs in the azaphosphine (II), which, even when heated for 9 hours under reflux with hydriodic acid, gave only the hydriodide of the unchanged phosphine.

The azaphosphine (II) readily combines with iodine in benzene to give a tri-iodide, which forms chocolate-brown crystals, m. p. 255—257°, and can be recrystallised unchanged from ethanol or benzene. This compound is insoluble in boiling water and even in boiling sodium hydrogen carbonate solution; its aqueous ethanolic solution contains no ionic iodine. It is immediately converted by sodium hydroxide into the azaphosphine oxide (VIII), and by cold sulphurous acid into the lemon-yellow crystalline hydriodide of the azaphosphine oxide; this salt is hydrolysed by cold aqueous sodium hydrogen carbonate to the oxide (VIII), which in turn when treated with hydriodic acid regenerates the yellow hydriodide. The precise structure of this hydriodide remains uncertain; the hydriodic acid may have neutralised the tertiary amine group of the phosphine oxide, or (more probably) have united with the P:O bond of the latter to give (IV; R = OH, X = I). It is clear from these reactions, however, that two of the iodine atoms of the original tri-iodide must be linked to the phosphorus atom; the position and mode of linkage of the third iodine atom also remain uncertain.

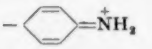
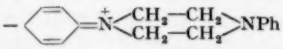
The ultra-violet absorption spectra of the piperazine (III) and of the azaphosphine and azarsine are shown in the Figure. Apart from the strong principal band due to the phenyl

groups which all the spectra show, it is of interest to note that the piperazine (III) shows two small secondary bands at 278—280 ($\varepsilon_{max.}$ 3000) and 282—284 mμ ($\varepsilon_{max.}$ 2990); the azaphosphine shows a slight inflexion at 310 mμ ($\varepsilon_{max.}$ 1000), whereas the azarsine shows a marked secondary band at 295—302 mμ ($\varepsilon_{max.}$ 1750). Aniline has a secondary band at 280 mμ ($\varepsilon_{max.}$ 1430) (Doub and Vandenbelt, *J. Amer. Chem. Soc.*, 1947, **69**, 2714), which has been attributed to polar forms such as (X) and analogous canonical forms. It is possible that the secondary bands in 1 : 4-diphenylpiperazine are due to similar polar forms such as (XI) (where charge separation can occur at one or both of the nitrogen atoms), and that the broader inflexions at greater wave-length shown by the azaphosphine and (more strongly) by the azarsine arise from a similar cause. This point could probably be determined if stable salts of the piperazine with two equivalents of acid, or if




*Absorption spectra of:*
*A*, 1 : 4-*Diphenylpiperazine* (mμ *scale at top of Figure*).
*B*, *Hexahydro*-1 : 4-*diphenyl*-1 : 4-*azaphosphine* (mμ *scale at base of Figure*).
*C*, *Hexahydro*-1 : 4-*diphenyl*-1 : 4-*azarsine* (mμ *scale at base of Figure*).

suitable diquaternary salts of the piperazine or the azarsine were available, since in such derivatives charge separation of the type shown in (XI) would not be possible. We find

(X)   (XI)

that 1 : 4-diphenylpiperazine dihydrochloride is too strongly dissociated in dilute solution to give reliable results, and diquaternary salts of the piperazine and the azarsine are at present unknown. In the case of the azaphosphine, the above inflexion is already so slight that investigation of its diquaternary salts for this purpose would probably prove fruitless.

Tests carried out in the Smith, Kline, and French Laboratories, Philadelphia, U.S.A., under the direction of Dr. Glenn E. Ullyot, show that the azaphosphine (II), when administered orally to rats in doses of 50, 100, and 150 mg./kg., failed to afford protection against histamine-induced bronchospasm.

We are now investigating the application of phenylphosphinebis(magnesium bromide) for the synthesis of other types of heterocyclic derivatives of phosphorus.

## Experimental

The azaphosphine and all its derivatives, unless otherwise stated, were colourless.

*Phenylphosphine.*—Reduction of phenyldichlorophosphine to phenylphosphine in 25% yield by lithium aluminium hydride has been described by Horvat and Furst (*J. Amer. Chem. Soc.*, 1952, **74**, 562). We find Michaelis and Kohler's original method (*Ber.*, 1877, **10**, 807), in which the dichlorophosphine is treated with ethanol, to be more satisfactory. This method apparently entails three distinct reactions. First, the dichlorophosphine is hydrolysed to phenylphosphonous acid: $Ph{\cdot}PCl_2 + EtOH = Ph{\cdot}P(OH)_2 + 2EtCl$. Secondly, the acid

when heated to *ca.* 250° undergoes thermal decomposition, forming phenylphosphonic acid and phenylphosphine: $3Ph{\cdot}P(OH)_2 = 2Ph{\cdot}PO_3H_2 + Ph{\cdot}PH_2$. Thirdly, the phosphonic acid on stronger heating decomposes to phosphoric acid and benzene: $Ph{\cdot}PO_3H_2 = HPO_3 + C_6H_6$. The second reaction is, however, strongly exothermic; consequently if suitable quantities are employed, it proceeds, when once started, spontaneously but quietly; the use of larger quantities, however, may lead to an uncontrollable reaction, whereas smaller quantities require external heating for distillation at this stage, with a considerable fall in yield. We find the following modification of Michaelis and Kohler's method probably represents optimum conditions.

Phenyldichlorophosphine (250 c.c.) was run dropwise into ethanol (600 c.c.); ethyl chloride was freely evolved, and external cooling was applied when necessary to the mixture. A stream of nitrogen was then led through the flask, and the excess of ethanol was removed under reduced pressure. The residual solution (which partly crystallised if allowed to cool) was transferred to a smaller distilling flask fitted with an inlet tube for nitrogen, and having a wide outlet tube, which was connected through an efficient condenser to a receiver; the latter in turn had an outlet tube, so that uncondensed vapour could be passed through permanganate–acetone solution. The whole preparation was performed in a vigorous draught. A slow stream of nitrogen was then led through the apparatus, and the solution was gently heated over a gauze; as soon as the vigorous reaction started, the flame was withdrawn. The phenylphosphine distilled rapidly, with only slight foaming; if necessary, the upper part of the flask was occasionally cooled to moderate the distillation. (When this reaction subsided, the flask was again heated and a second fraction, consisting mainly of benzene with very little phosphine and a high-boiling component, was collected; it was found advisable, however, to ignore this fraction, because the low phosphine content did not justify its laborious refractionation.) The first fraction was washed with water, dried ($CaCl_2$), and fractionally distilled in nitrogen. The early runnings of ethanol and benzene were followed by the phenylphosphine, b. p. 157—159° (yield: 36 g., 54%). A very small residue remained in the distillation flask.

The phosphine, which is rapidly oxidised in air, was sealed in weighed ampoules. In the following preparation and use of the Grignard reagent, the quantities of reagents were adjusted to that of the phosphine to be used, so that exposure of the latter to the air was reduced to a minimum. The phosphine when in benzene solution is much less readily oxidised than when pure.

*Hexahydro*-1 : 4-*diphenyl*-1 : 4-*azaphosphine* (II).—A Grignard reagent was prepared from bromobenzene (13·7 g., 2·3 mols.), magnesium (2·22 g., 2·4 atoms), and ether (90 c.c.) in a flask fitted with a stirrer, reflux condenser, dropping funnel, and an inlet tube through which nitrogen was passed throughout the experiment, the ethereal solution being finally boiled under reflux for 30 minutes to ensure completion of the preparation. The solution was then cooled and vigorously stirred, whilst a solution of phenylphosphine (4·2 g., 1 mol.) in benzene (40 c.c.) was run in during 15 minutes. The mixture was then boiled under reflux with stirring for 2·5 hours, benzene (30 c.c.) being later added to replace the ether carried out in the nitrogen stream. The solution of phenylphosphinebis(magnesium bromide) was cooled to 30° and di-2′-bromoethylaniline (9·4 g., 0·8 mol.) in benzene (30 c.c.) added with vigorous stirring during 30 minutes, the complete mixture being then boiled as before for 2 hours. It was finally cooled in ice-water and hydrolysed with aqueous ammonium chloride solution. The organic layer was separated, the aqueous layer (which tended to become semi-solid) was repeatedly extracted with benzene, and the united organic solutions, without filtration or drying, were concentrated under reduced pressure in nitrogen. The residue, which solidified considerably on cooling, was recrystallised from ethanol and afforded the *azaphosphine* (II), m. p. 89—90° (Found: C, 75·0; H, 7·1; N, 5·3%; *M*, ebullioscopic in 0·679% ethanol solution, 241. $C_{16}H_{18}NP$ requires C, 75·3; H, 7·1; N, 5·5%; *M*, 255); the yield was 4·3 g. (55%). The azaphosphine is slightly hygroscopic, and was dried at 60°/0·5 mm. for 4 hours before analysis.

The azaphosphine when dissolved in a minimum of cold concentrated hydrochloric acid rapidly deposited a heavily hydrated crystalline *dihydrochloride* which when dried in a vacuum over phosphoric anhydride gave the anhydrous salt, m. p. 165—166° (Found: C, 59·0; H, 6·5; N, 4·5. $C_{16}H_{18}NP,2HCl$ requires C, 58·5; H, 6·3; N, 4·3%). The azaphosphine also gave a crystalline *monohydriodide*, m. p. 190—191°, which became brown on exposure to light (Found: C, 49·9; H, 5·3; N, 3·5. $C_{16}H_{18}NP,HI$ requires C, 50·1; H, 5·0; N, 3·65%), and a *monopicrate*, which separated as orange crystals, m. p. 131—132° (preliminary softening), when hot ethanolic solutions of the phosphine and of an excess of picric acid were mixed and cooled (Found: C, 54·2; H, 4·5; N, 11·4. $C_{16}H_{18}NP,C_6H_3O_7N_3$ requires C, 54·5; H, 4·4; N, 11·6%).

*Dibromobis*(*hexahydro*-1 : 4-*diphenyl*-1 : 4-*azaphosphine*)*palladium*. When mixed ethanolic

solutions of the phosphine and of potassium palladobromide were boiled, this *compound* was precipitated and, when collected, washed with ethanol, and recrystallised from ethanolic benzene (1 : 1), gave orange-yellow crystals, m. p. 184—185° (Found: C, 49·4; H, 4·9; N, 3·3. $C_{32}H_{36}N_2Br_2P_2Pd$ requires C, 49·45; H, 4·7; N, 3·6%).

*Quaternary salts.* A solution of the azaphosphine in an excess of cold methyl bromide, when set aside in a sealed tube for 24 hours, deposited the crystalline *monomethobromide* (IV; R = Me, X = Br), which had m. p. 152° after being collected, washed with light petroleum (b. p. 60—80°), and dried (Found: C, 58·1; H, 6·2; N, 3·9. $C_{17}H_{21}NBrP$ requires C, 58·3; H, 6·1; N, 4·0%). No satisfactory solvent for recrystallisation could be found.

The *monomethobromide hydrobromide* separated when a solution of the azaphosphine (0·2 g.) in methyl bromide (2 c.c.) and methanol (2 c.c.) was heated in nitrogen in a sealed tube at 100° for 6 hours and then cooled. The salt, after recrystallisation from methanol, had m. p. 279—283° (decomp.) (Found: C, 47·5; H, 5·5; N, 3.3. $C_{17}H_{21}NBrP,HBr$ requires C, 47·35; H, 5·15; N, 3·25%).

Both these salts, when treated in aqueous solution with sodium picrate, deposited the *monomethopicrate* (IV; R = Me, X = $C_6H_2O_7N_3$), which crystallised from water in orange-red crystals, m. p. 118° (Found: C, 55·35; H, 5·1; N, 11·1. $C_{23}H_{23}O_7N_4P$ requires C, 55·4; H, 4·65; N, 11·2%).

A solution of the azaphosphine in an excess of cold ethyl bromide, when either set aside at room temperature for 3 days or heated in a sealed tube at 100° for 6 hours, deposited crystals of the *monoethobromide* (IV; R = Et, X = Br), m. p. 175—176° (Found: C, 59·2; H, 6·6; N, 4·1. $C_{18}H_{23}NBrP$ requires C, 59·3; H, 6·4; N, 3·85%). This salt also could not be recrystallised.

The *monoethobromide hydrobromide* was prepared (*a*) by heating the azaphosphine in ethanolic ethyl bromide at 100° for 6 hours or (*b*) by the addition of hydrobromic acid to a cold concentrated aqueous solution of the monoethobromide. It was recrystallised from methanol or hydrobromic acid, and had m. p. 278—284° (decomp.) (Found: C, 48·6; H, 5·4; N, 3·3; Br, 36·7. $C_{18}H_{23}NBrP,HBr$ requires C, 48·55; H, 5·4; N, 3·15. Br, 35·9%). When this salt in aqueous solution was treated with aqueous sodium hydroxide (1 mol.), the monoethobromide, m. p. 174° (alone and mixed), was recovered.

The azaphosphine dissolved in methyl iodide with considerable evolution of heat: the residue, when recrystallised from acetone–light petroleum (b. p. 60—80°) gave the *monomethiodide* (IV; R = Me, X = I), m. p. 155—156° (Found: C, 51·1; H, 5·1; N, 3·7. $C_{17}H_{21}NIP$ requires C, 51·4; H, 5·3; N, 3·5%). A methanolic solution treated with sodium picrate gave the above monomethopicrate, m. p. 118° (alone and mixed).

The azaphosphine or its monomethiodide, when boiled under reflux with methyl iodide, readily deposited the *dimethiodide* (V; R = R′ = Me, X = I), m. p. 118° after recrystallisation from methanolic acetone (Found: C, 39·6; H, 4·7; N, 2·7. $C_{18}H_{24}NI_2P$ requires C, 40·1; H, 4·5; N, 2·6%). This salt was highly hygroscopic, but drying in a vacuum even at very moderate temperatures caused loss of methyl iodide.

When aqueous solutions of this salt and of sodium picrate were mixed, the *dimethopicrate* was precipitated, and when collected, washed with water, and recrystallised from dioxan formed yellow crystals, m. p. 172—174° (Found: C, 48·7; H, 3·85; N, 13·1. $C_{30}H_{28}O_{14}N_7P$ requires C, 48·6; H, 3·8; N, 13·2%).

A mixture of the azaphosphine and an excess of methyl toluene-*p*-sulphonate was heated at 100° for 6 hours in a sealed tube, and the cold syrupy product, when thoroughly washed with ether, left a residue of the crystalline dimethotoluene-*p*-sulphonate. This was highly deliquescent and could not be recrystallised; it was therefore characterised by conversion into the above dimethopicrate, m. p. 172—174° (alone and mixed).

s-*Ethylenebis*(*hexahydro*-1 : 4-*diphenyl*-1 : 4-*azaphosphonium*) *Dibromide* (VI).—A mixture of the azaphosphine (0·51 g.) and pure dry ethylene dibromide (0·38 g., 1 mol.) was heated under nitrogen in a sealed tube at 130—135° for 7 hours. Unchanged ethylene dibromide was then removed at 100°/14 mm., and the greyish-brown residue, m. p. 240—245°, extracted with boiling ethanol (15 c.c.) to remove coloured impurities. The almost white residue was then thrice recrystallised from more ethanol, and the *dibromide* (VI) obtained as the crystalline *monoethanolate*, m. p. 273—274° (Found: C, 57·85; H, 6·0; N, 4·0. $C_{34}H_{40}N_2Br_2P_2,C_2H_6O$ requires C, 58·05; H, 6·2; N, 3·8%).

Other experiments in which the azaphosphine was heated with ethylene dibromide (1·1 mols.) at 150—160° for 6 hours, and with the dibromide (1·5 mols.) at 135—145° for 6 hours, gave the same product.

The dibromide (VI) was recovered unchanged after it had been boiled under reflux with an excess of methyl iodide for 30 minutes. When treated in aqueous solution with aqueous sodium picrate, the dibromide (VI) deposited the *dipicrate*, mustard-yellow crystals, m. p. 190—191°, from ethanol (Found : C, 55·3; H, 4·7; N, 11·2. $C_{46}H_{44}O_{14}N_8P_2$ requires C, 55·5; H, 4·5; N, 11·25%).

When the azaphosphine was heated with trimethylene dibromide (1 mol.) in a sealed tube for 6 hours at 140—145°, or at 170—175°, the product was a pale amber glass which could not be crystallised and did not yield a homogeneous picrate.

The structures of the azaphosphine derivatives described above show clearly however that, when the phosphine group in (II) has undergone salt formation, either by direct union with acids or by quaternisation, the tertiary amine group becomes partly deactivated, and although it can still form reasonably stable salts with strong acids such as hydrochloric and hydrobromic acid, it cannot do so with weak acids such as hydriodic and picric acids. Many similar examples of partial deactivation have been given by Mann and Watson (*J. Org. Chem.*, 1948, **13**, 502).

*The Azaphosphine Monoxide* (VIII).—(*a*) A solution of the azaphosphine (0·2 g.) in acetone (10 c.c.) became warm when diluted with 3% aqueous hydrogen peroxide (15 c.c.); it was next set aside for 24 hours and then evaporated to small bulk, and on cooling deposited the crystalline *monoxide*, m. p. 145—147° after recrystallisation from water (Found : C, 70·5; H, 6·45; N, 5·4. $C_{16}H_{18}ONP$ requires C, 70·8; H, 6·7; N, 5·2%).

When the acetone solution of the azaphosphine was diluted with an equal volume of 30% hydrogen peroxide and heated at 70—80° for 8 hours, the above monoxide, m. p. 143—144° (alone and mixed), was again obtained.

(*b*) A solution of the azaphosphine (0·255 g.) and chloramine-T (0·28 g., 1 mol.) in ethanol (35 c.c.) was boiled under reflux for 1 hour, and then concentrated by distillation. Addition of dilute potassium hydroxide to the cold solution precipitated a gum which when recrystallised from water gave the monoxide, m. p. 145° (alone and mixed).

The monoxide was unaffected by exposure to air. Its aqueous solution when treated with picric acid deposited the yellow *hydroxypicrate*, m. p. 165—167° (preliminary darkening) after recrystallisation from water (Found : C, 52·7; H, 4·2; N, 11·0. $C_{32}H_{21}O_8N_4P$ requires C, 52·8; H, 4·2; N, 11·2%). A solution of the monoxide in concentrated hydrochloric acid, when evaporated in a vacuum at room temperature, deposited crystals of the *hydroxychloride-hydrochloride*, m. p. 225° after drying in a vacuum over phosphoric anhydride for 5 days (Found : C, 54·8; H, 6·1; N, 4·1. $C_{16}H_{18}ONP,2HCl$ requires C, 55·8; H, 5·9; N, 4·1%). The low carbon value is due to the fact that the salt was too hygroscopic to allow recrystallisation, and drying in a vacuum even at 40° caused partial loss of hydrogen chloride.

*The Azaphosphine Dioxide* (IX).—A solution of the azaphosphine in acetic acid was diluted with an equal volume of 30% hydrogen peroxide, heated at 70—80° for 7 hours, and then evaporated, first at 70° and later at room temperature in a vacuum. The residual syrup, when recrystallised from ethanol, gave the *monohydrated dioxide–hydrogen peroxide* addition compound, m. p. 149° (vigorous effervescence with formation of a yellow liquid) after heating at 50°/0·5 mm. for 6 hours over phosphoric anhydride (Found : C, 56·7; H, 6·1; N, 4·5. $C_{16}H_{18}O_3NP,H_2O_2,H_2O$ requires C, 56·6; H, 6·55; N, 4·1%). An aqueous solution of the compound is neutral, and gives no precipitate with sodium hydroxide but an immediate blue colour with potassium iodide–starch.

*Action of Hydriodic Acid on the Azaphosphine.*—A mixture of the azaphosphine (0·2 g.) and pure hydriodic acid of constant b. p. (25 c.c.) was boiled under reflux in nitrogen for 9 hours, and then concentrated under reduced pressure, the crystalline azaphosphine hydriodide, identical in properties with that previously described, separating.

*The Azaphosphine Tri-iodide.*—(*a*) Hot benzene solutions of the azaphosphine and of iodine, when mixed and cooled, deposited chocolate brown crystals of the *tri-iodide*, m. p. 255—257° (decomp.) after recrystallisation from ethanol (Found : C, 30·5; H, 2·9; N, 2·2. $C_{16}H_{18}NI_3P$ requires C, 30·2; H, 2·9; N, 2·2%).

(*b*) When solutions of the azaphosphine and of iodine, each in hydriodic acid, were mixed and set aside, the tri-iodide slowly separated.

When aqueous sodium hydroxide was added to a hot aqueous suspension of the tri-iodide, a clear solution was at once obtained, and on cooling deposited the crystalline monoxide, m. p. 143—144° (alone and mixed).

When an aqueous solution of sulphur dioxide was added to a cold aqueous suspension of the tri-iodide, the latter was rapidly converted into the lemon-yellow crystalline *azaphosphine oxide hydriodide*, which, when collected, washed with sulphurous acid, and dried in vacuum at

room temperature, had m. p. 257—260° (preliminary softening) (Found: C, 47·8; H, 4·8; N, 3·4. $C_{16}H_{18}ONP,HI$ requires C, 48·1; H, 4·8; N, 3·5%). This salt was precipitated when hydriodic acid was added to a cold saturated solution of the azaphosphine monoxide. It is soluble in cold water, and its aqueous solution when either boiled for a few minutes, or treated with cold aqueous sodium hydrogen carbonate, deposited the monoxide, m. p. 144—145° (alone and mixed).

Since Beeby and Mann (*loc. cit.*) obtained the monomethiodide of the azarsine (I) by boiling with methyl iodide, it was clearly desirable to determine whether a dimethiodide would be formed under the more vigorous conditions employed to prepare the azaphosphine dimethiodide. A solution of the azarsine in an excess of methyl iodide was therefore heated under nitrogen in a sealed tube at 100° for 8 hours. The product, however, yielded only the former monomethiodide, m. p. 181° (alone and mixed) (Found: N, 3·2. Calc. for $C_{17}H_{21}NIAs$: N, 3·2%).

1 : 4-*Diphenylpiperazine Dihydrochloride*.—This salt, which was briefly described without adequate characterisation by Hofmann (*Proc. Roy. Soc.*, 1858, **9**, 277), is readily prepared by passing hydrogen chloride into a warm ethereal solution of the piperazine (III); the precipitated dihydrochloride, when recrystallised from ethanol containing a small proportion of concentrated hydrochloric acid, forms colourless crystals, m. p. 222—223° (Found: N, 9·2. Calc. for $C_{16}H_{18}N_2,2HCl$: N, 9·0%). Contrary to Hofmann's statement, the dry salt is stable, but when boiled with water gives the free base.

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## 582. *The Preparation of Bis-2-cyanoethyl Derivatives of Aromatic Primary Amines, and their Conversion into* 1 : 6-*Diketojulolidines.*

By JOHN T. BRAUNHOLTZ and FREDERICK G. MANN.

The conditions are described for the conversion of certain aromatic primary amines into their bis-2-cyanoethyl derivatives, and for the cyclisation of the latter to the corresponding 1 : 6-diketojulolidines. The properties of the diketo-compounds have been investigated; the dioxime of the unsubstituted 1 : 6-diketojulolidine shows unexpected stability.

IT has been shown by R. C. Cookson and Mann (*J.*, 1949, 67) that heating aniline with an excess of vinyl cyanide in acetic acid at 150° gives a mixture of *N*-2-cyanoethylaniline, $Ph{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CN$, and *NN*-bis-2-cyanoethylaniline, $Ph{\cdot}N(CH_2{\cdot}CH_2{\cdot}CN)_2$. This is apparently the first recorded example of an aromatic primary amine undergoing dicyanoethylation, although Elderfield, Gensler, Bembry, Kremer, Brody, Hageman, and Head (*J. Amer. Chem. Soc.*, 1946, **68**, 1262) had shown that *p*-anisidine under similar conditions gave the monocyanoethyl derivative.

We have therefore investigated further the reaction of aromatic primary amines with vinyl cyanide, and find that the production of the biscyanoethyl derivative is apparently a general process. Our results are summarised in the following Table, in which are recorded the products obtained by heating various amines with vinyl cyanide (2·5 mols.) and acetic acid (2·4—2·5 mols.) at 140—145° for 3—6 hours.

| Amine | Time of heating (hours) | Mono-deriv. $R{\cdot}NH{\cdot}C_2H_4{\cdot}CN$ M. p. | Mono-deriv. Yield, % | Di-deriv. $R{\cdot}N(C_2H_4{\cdot}CN)_2$ M. p. | Di-deriv. Yield, % |
|---|---|---|---|---|---|
| Aniline | 3·5 | 51·5° | 32 | 80—82° | 13 |
| *m*-Toluidine | 4·5 | 47·5—48·5 | 50 | 85—86 | 32 |
| *p*-Toluidine | 6 | 103—104 | 54 | 61—62 | 42 |
| *p*-Anisidine | 5·5 | 64 [1] | — | 100—101 | 52 |
| *p*-Chloroaniline | 3·25 | 76 [2] | — | 91 | 4 |

[1] Elderfield *et al.* (*loc. cit.*). [2] Bauer, Cymerman, and Sheldon, *J.*, 1951, 3312; these mono-derivatives were not isolated in our experiments.

Although the experiments have been repeated many times, it is not claimed that the yields given in the Table (which represent those of the crude material once recrystallised) could not be further improved. It is noteworthy that the addition of a mixture of copper powder and copper acetate to the reaction mixture (cf. B.P. 457,621) did not appreciably

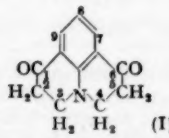
affect the yield of the products from *m*-toluidine and *p*-chloroaniline: furthermore, *p*-toluidine when heated with vinyl cyanide, formic acid, copper powder, and copper formate gave only the monocyanoethyl derivative, and when heated with vinyl cyanide, *cyclo*hexane, and a trace of sodium methoxide remained unaffected. These observations indicate that the probable function of the acetic acid is to form the ion, $R{\cdot}NH_3^+$, which is apparently more susceptible to cyanoethylation than the free base. The other possibility, namely, that acetanilide (or its analogues) is the reactive intermediate, is disproved, because we have found that acetanilide, after being heated with vinyl cyanide and acetic acid under conditions similar to those cited in the preceding paragraph, was recovered in almost theoretical quantity.

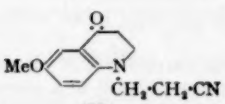
Certain properties of the biscyanoethyl derivatives have been investigated. It will be noted that, with one exception, all have melting points above those of the corresponding monocyanoethyl derivatives: no explanation of the exception (*p*-toluidine) is apparent.

It has been pointed out (R. C. Cookson and Mann, *J.*, 1947, 618; Mann and Watson, *J. Org. Chem.*, 1948, **13**, 502) that the inductive effect of the cyano-groups in bis-2-cyanoethylphenylarsine, $Ph{\cdot}As(CH_2{\cdot}CH_2{\cdot}CN)_2$, partly suppresses the normal reactivity of the tertiary arsine, which, for example, is stable to air for long periods, but gives a quaternary salt in the presence of boiling methyl iodide. A similar partial inactivation of the nitrogen atom occurs in bis-2-cyanoethylaniline. This forms a crystalline hydrochloride and a chloroplatinate, but these when mixed with water immediately deposit the free base. On the other hand it does not apparently form a picrate, and does not combine with boiling methyl iodide or molten methyl toluene-*p*-sulphonate: it is also unaffected by 30% hydrogen peroxide. These properties are in marked contrast to those of *N*-2-cyanoethyl-*N*-methylaniline, $Ph{\cdot}NMe{\cdot}CH_2{\cdot}CH_2{\cdot}CN$, in which the inductive effect of the cyano-group upon the tertiary nitrogen atom is necessarily much weaker; the amine consequently forms a picrate (Whitmore, Mosher, Adams, Taylor, Chapin, Weisel, and Yanko, *J. Amer. Chem. Soc.*, 1944, **66**, 725) and we find that it also readily forms a methiodide.

Bis-2-cyanoethylaniline and its *m*-toluidine homologue behave as typical dialkylarylamines in that they form green crystalline *p*-nitroso-derivatives.

Mann and Smith (*J.*, 1951, 1898) have shown that bis-2-cyanoethylaniline when heated with aluminium chloride in chlorobenzene undergoes cyclisation to 1 : 6-diketojulolidine (I). We have both extended the scope of this reaction and investigated certain properties of the diketone (I). Bis-2-cyanoethyl-*m*-toluidine undergoes ready cyclisation to 1 : 6-diketo-7-methyljulolidine, but the *p*-toluidine analogue gives the 8-methyldiketone in only 7% yield, and the *p*-anisidine analogue undergoes cyclisation and demethylation to give 8-hydroxy-1 : 6-diketojulolidine, also in 7% yield. All these diketojulolidines are crystalline and readily give crystalline but almost insoluble bisphenylhydrazones.


(I)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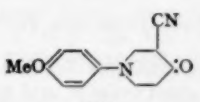
(III)

Solutions of the diketones in polar solvents show marked fluorescence, ranging from yellow-green to orange, which, however, is much feebler in solvents such as benzene and *cyclo*hexane. It has been claimed (F.P. 806,715) that cyclisations of the above type can be carried out by heating appropriate nitriles with a mixture of aluminium chloride, potassium chloride, and sodium chloride, but this method proved useless for our purpose. The production of 1 : 6-diketo-7-methyl- and 8-hydroxy-1 : 6-diketo-julolidine was accompanied by that of corresponding monoketones, the structures of which are discussed below.

The diketone (I) also readily gives a crystalline dioxime, which possesses unexpected stability: we have failed to reduce it by sodium and methanol, sodium amalgam, lithium aluminium hydride, or hydrogen and a palladium–carbon catalyst. During these experiments we have isolated the dibenzoate, ditoluene-*p*-sulphonate, and diacetate of the dioxime. The first of these derivatives shows a remarkable variation in melting-point: one sample

when thrice recrystallised from ethanol–acetone had m. p.s 172°, 186°, and 175·5°; another sample, recrystallised from ethanol–acetone, had m. p. 204°, depressed to 201° by admixture with the sample of m. p. 175·5°. The dioxime and its esters can theoretically exist as *syn-*, *anti-*, and *amphi*-forms, and it is possible that the above variation may be due to a very ready interconversion of two or three of these isomeric forms. Furthermore, the dioxime ditoluene-*p*-sulphonate, when analytically pure, began to melt at 145° but was not completely molten at 200°, possibly owing to its being a mixture of isomeric forms or to the slow conversion of one form into another; on the other hand, it may have been due to the onset of the Beckmann rearrangement, for Kuhara ("On the Beckmann Rearrangement," Imp. Univ. Kyoto, Tokyo, 1926; cf. also Chapman, *J.*, 1933, 806; 1934, 1550; 1936, 448) has shown that the benzenesulphonate esters of oximes may undergo the Beckmann rearrangement spontaneously at room temperature, and rapidly when heated. We hope later to examine these esters more fully.

When, however, the cyclisation of bis-2-cyanoethyl-*m*-toluidine was performed with a smaller proportion of aluminium chloride, a pale yellow crystalline monoketone, $C_{13}H_{14}ON_2$, was isolated in small yield: bis-2-cyanoethyl-*p*-anisidine similarly gave a monoketone, $C_{13}H_{14}O_2N_2$, which, unlike the diketone, still retained the methoxyl group intact. It will be clear that the *p*-methoxyphenyl monoketone, for example, might be either 1-2′-cyanoethyl-1 : 2 : 3 : 4-tetrahydro-6-methoxyquinol-4-one (II) or the isomeric 3-cyano-1-*p*-methoxyphenylpiperid-4-one (III). The small amount of these monoketones available precluded a ready chemical identification of their structure. It is a reasonable assumption, however, that the ultra-violet absorption of (III) should be not markedly different from that of the parent compound, $MeO{\cdot}C_6H_4{\cdot}N(CH_2{\cdot}CH_2{\cdot}CN)_2$, whereas that of the quinolone (II) would undoubtedly show considerable differences, particularly as the carbonyl group is now directly conjugated to the aromatic ring. The spectrum of the parent dinitrile in ethanol shows maxima at 312 ($\varepsilon_{max.}$ 1830) and 251 mμ ($\varepsilon_{max.}$ 11,600) with minima at 277 ($\varepsilon_{min.}$ 795) and 220 mμ ($\varepsilon_{min.}$ 4860). That of the monoketone, also in ethanol, shows maxima at 410 ($\varepsilon_{max.}$ 5070), 264 ($\varepsilon_{max.}$ 7760) and 239 mμ ($\varepsilon_{max.}$ 22,400), with minima at 297 ($\varepsilon_{min.}$ 100) and 259 mμ ($\varepsilon_{min.}$ 7460). These wide differences make it highly probable that the monoketone is the quinolone (II). This receives some chemical suppport in the *p*-methoxyphenyl series from the fact that the action of the aluminium chloride is clearly milder in the formation of the monoketone than in that of the diketone: consequently the methoxyl group is not demethylated and apparently only one of the 2-cyanoethyl groups undergoes cyclisation. Quinol-4-ones of type (II) would be of great value, because hydrolysis of the nitrile group followed by decarboxylation would yield the 1-alkyltetrahydroquinol-4-ones, which are otherwise difficult to prepare.

## Experimental

Certain compounds gave sharp m. p.s only in an evacuated tube; these are denoted by (E.T.).

*Cyanoethylation.*—This process was carried out in all cases by heating the mixture of the amine, vinyl cyanide, and acetic acid (see p. 3046) in a glass tube which formed the lining of a stainless-steel autoclave. Only the treatment of the product is therefore described below.

*Aniline.* Cyanoethylation was performed under conditions similar to those of R. C. Cookson and Mann (*loc. cit.*), and distillation of the higher-boiling material gave two fractions: (*a*), b. p. 120—130°/0·35 mm., gave 2-cyanoethylaniline (32%), m. p. 49—50° after one recrystallisation from aqueous ethanol; (*b*), b. p. 180—190°/0·35 mm., similarly recrystallised, gave bis-2-cyanoethylaniline (13%), m. p. 79—80°.

Dry hydrogen chloride was passed into a cold acetone solution of bis-2-cyanoethylaniline, which, when subsequently diluted with ether, deposited the white crystalline *hydrochloride*, m. p. 115·5—116·5° after being washed with dry ether and dried over calcium chloride (Found: N, 17·9. $C_{12}H_{13}N_3,HCl$ requires N, 17·9%). A solution of amine in warm concentrated hydrochloric acid was added to concentrated aqueous chloroplatinic acid: the mixture when cooled deposited orange-brown crystals of the *chloroplatinate*, which when washed with ice-cold concentrated hydrochloric acid and dried in a vacuum-desiccator, had m. p. 148—149° (decomp.) [Found: C, 35·2; H, 3·6; N, 10·3; Pt, 24·1. $(C_{12}H_{13}N_3)_2,H_2PtCl_6$ requires C, 35·6; H, 3·5; N, 10·4; Pt, 24·1%].

Bis-2-cyanoethylaniline was recovered unchanged after (*a*) its solution in an excess of methyl iodide had been heated at 100° in a sealed tube for 14 hours, (*b*) its solution in methyl iodide containing nitromethane had been boiled under reflux for 1·25 hours, (*c*) a mixture with an excess of methyl toluene-*p*-sulphonate had been heated at 165° for 30 minutes, and (*d*) a solution in acetone containing an excess of 30% hydrogen peroxide had been set aside at room temperature for 15 hours.

NN-*Bis*-2-*cyanoethyl*-p-*nitrosoaniline*, prepared in the usual way, formed shining green plates (from ethanol), m. p. 138—139° (Found: C, 63·4; H, 5·35; N, 24·3. $C_{12}H_{12}ON_4$ requires C, 63·2; H, 5·3; N, 24·5%).

m-*Toluidine*. The crude cyanoethylation product, a dark brown viscous liquid, was first heated at 18 mm. to remove low-boiling constituents; the residue when distilled at 0·2 mm. gave the fractions: (*a*), b. p. 125—150°, which slowly solidified, (*b*), b. p. 160—190°, which apidly solidified. Fraction (*a*), after recrystallisation from ethanol, gave 2-*cyanoethyl*-m-*toluidine*, colourless plates, m. p. 47·5—48·5° (50%) (Found: C, 75·2; H, 7·5; N, 17·35. $C_{10}H_{12}N_2$ requires C, 75·0; H, 7·5; N, 17·5%). Fraction (*b*), after repeated recrystallisation from ethanol, gave *bis*-2-*cyanoethyl*-m-*toluidine*, colourless needles, m. p. 85—86° (32%) (Found: C, 73·3; H, 7·3; N, 19·9. $C_{13}H_{16}N_2$ requires C, 73·2; H, 7·0; N, 19·7%).

Treatment with nitrous acid in the usual way gave NN-*bis*-2′-*cyanoethyl*-4-*nitroso*-m-*toluidine* ($NH_2 = 1$), dark green plates (from ethanol), m. p. 157—158° (Found: C, 64·4; H, 5·8; N, 22·9. $C_{13}H_{14}ON_4$ requires C, 64·4; H, 5·8; N, 23·1).

p-*Toluidine*. The crude product, when treated as above, also gave two fractions on distillation at 0·2 mm., both rapidly solidifying. Fraction (*a*), b. p. 140—180°, recrystallised from ethanol, gave N-2-*cyanoethyl*-p-*toluidine*, colourless plates, m. p. 103—104° (54%) (Found: C, 75·2; H, 7·5; N, 17·3%). Fraction (*b*), b. p. 180—205°, similarly recrystallised, gave NN-*bis*-2-*cyanoethyl*-p-*toluidine*, colourless needles, m. p. 61—62° (42%) (Found: C, 73·05; H, 7·1; N, 20·1%).

When a mixture of *p*-toluidine (11 g.), vinyl cyanide (17·5 c.c., 2·6 mols.), *cyclo*hexane (15 c.c.), and sodium methoxide (0·5 g.) was heated at 140—150° for 3 hours, the product showed evidence of polymerisation, and distillation at 0·5 mm. yielded unchanged *p*-toluidine (8·5 g., 77%) and no cyanoethyl derivatives.

When a mixture of *p*-toluidine (11 g.), vinyl cyanide (20 c.c., 3 mols.), formic acid (10 c.c.), copper powder (1 g.), and copper formate (0·5 g.) was heated at 180—190° for 3 hours, distillation gave unchanged *p*-toluidine, a small yield of the above fraction (*a*), and a very small higher-boiling fraction.

p-*Anisidine*. The cold reaction product solidified in all experiments except the first, and when recrystallised from ethanol gave NN-*bis*-2-*cyanoethyl*-p-*anisidine*, colourless crystals, m. p. 100—101°, b. p. 214—218°/0·2 mm. (52%) (Found: C, 68·3; H, 6·4; N, 18·5. $C_{13}H_{16}ON_2$ requires C, 68·1; H, 6·55; N, 18·3%).

p-*Chloroaniline*. The crude reaction product was distilled at 0·1 mm. after low-boiling constituents had been removed, and furnished (*a*) unchanged *p*-chloroaniline, (*b*) *p*-chloroacetanilide, m. p. 175—176° (Found: N, 8·15. Calc. for $C_8H_8ONCl$: N, 8·3%), (*c*) a yellow oil, b. p. 180—210°, which slowly solidified and when recrystallised from ethanol gave p-*chloro*-NN-*bis*-2-*cyanoethylaniline*, colourless needles, m. p. 91° (4%) (Found: C, 62·0; H, 5·1; N, 17·9. $C_{12}H_{12}N_2Cl$ requires C, 61·7; H, 5·1; N, 18·0%). No indication of the monocyanoethylamine was obtained.

*Acetanilide*. (*a*) When a mixture of acetanilide (3 g.), vinyl cyanide (1·9 c.c., 1·25 mols.), and acetic acid (1·65 c.c., 1·25 mols.) was similarly heated at 140° for 14 hours, the straw-coloured viscous liquid on cooling deposited unchanged acetanilide in almost theoretical yield. (*b*) An identical result was obtained when a mixture of acetanilide (22·5 g.), vinyl cyanide (18·8 c.c.), acetic acid (12·5 c.c.), and water (3 c.c.) was heated at 142° for 4·5 hours. The water added to this mixture was equivalent to that which would have been formed in the cyanoethylation of the primary amines had they undergone acetylation as an intermediate stage in cyanoethylation process.

N-2-*Cyanoethyl*-NN-*dimethylanilinium Iodide*.—This salt readily crystallised when a solution of 2-cyanoethyl-*N*-methylaniline in an excess of methyl iodide was boiled under reflux for 1 hour and cooled; recrystallisation from ethanol gave the colourless *iodide*, m. p. 126° (Found: C, 43·8; H, 5·1; N, 9·4. $C_{11}H_{15}N_2I$ requires C, 43·7; H, 5·0; N, 9·3%).

1:6-*Diketojulolidine* (I). This was prepared by the following modification of Mann and Smith's method (*loc. cit.*). Bis-2-cyanoethylaniline (9 g.) was added to a mixture of powdered aluminium chloride (40 g., 6·7 mols.), chlorobenzene (26 c.c.), and concentrated hydrochloric acid (1·5 c.c.),

which was then heated at 150° for 7·5 hours with vigorous stirring. The red oily mixture was then cooled, hydrolysed with ice-water, and distilled in steam to remove chlorobenzene. The aqueous residue (*ca.* 150 c.c.), when allowed to cool slowly, deposited the crude diketone, which when once recrystallised from ethanol had m. p. 134—136° (yield 8 g., 89%); this material was not quite pure, however, because Mann and Smith (*loc. cit.*) gave m. p. 145—146°. Consistent yields in this and analogous preparations described below were obtained only if aluminium chloride of high purity were used, and if the hydrolysis was carefully performed to prevent any appreciable rise in temperature.

*The Dioxime of* (I).—A mixture of the diketone (0·25 g.) and a solution of hydroxylamine hydrochloride (2 g.) in 10% aqueous sodium carbonate (24 c.c.) and ethanol (5 c.c.) was boiled under reflux for 3 hours, and on cooling deposited the *dioxime* as pale yellow needles (0·2 g., 70%) which when recrystallised from aqueous ethanol had m. p. 237·5—238·5° (decomp.) (Found: C, 62·5; H, 5·3; N, 18·3. $C_{12}H_{13}O_2N_3$ requires C, 62·3; H, 5·7; N, 18·2%).

When the dioxime was shaken with an excess of benzoyl chloride in 10% aqueous sodium hydroxide suspension, the *dibenzoate* was readily obtained and after successive recrystallisations from ethanol–acetone had m. p. 172°, 186°, 175·5° (decomp. throughout); the final product was analysed (Found: N, 9·7. $C_{26}H_{21}O_4N_3$ requires N, 9·6%).

*Attempted reduction.* (*a*) Sodium (4·5 g., 16·5 atomic proportions) was added during 1·5 hours to a boiling solution of dioxime (1·4 g.) in methanol (50 c.c.). The solution was then distilled in steam, superheated steam being finally used, but the collected distillate (400 c.c.) when acidified with hydrochloric acid and evaporated gave no residue. The strongly alkaline residual solution was then divided into two portions. (i) One portion was vigorously shaken for 1 hour with an excess of benzoyl chloride, and the dibenzoate of the dioxime obtained as a fine yellow crystalline powder, which when thrice recrystallised from ethanol–acetone had m. p. 143°, 193°, 204° (decomp. throughout); the last sample was analysed (Found: C, 70·8; H, 4·7; N, 9·6. Calc. for $C_{26}H_{21}O_4N_3$: C, 71·1; H, 4·8; N, 9·6%). A mixture of the sample of m. p. 204° with that of m. p. 175·5° obtained directly had m. p. 201°. (ii) The second portion was similarly treated with an excess of toluene-*p*-sulphonyl chloride and gave the *ditoluene-p-sulphonate* of the dioxime, which after two recrystallisations from ethanol–acetone formed pale yellow needles, which softened at 154° but were not completely molten at 200°: after a third recrystallisation they melted similarly from 145° to *ca.* 200° (Found: for material twice recrystallised, N, 7·8; thrice recrystallised, C, 58·0; H, 4·8; N, 7·7. $C_{26}H_{25}O_6N_3S_2$ requires C, 57·9; H, 4·7; N, 7·8%). The crystals darkened slowly on exposure to light and air.

(*b*) A solution of the dioxime (1 g.) in methanol (15 c.c.) was treated with 2·5% sodium amalgam (100 g.) and acetic acid in alternate portions during 2 hours so that the solution remained slightly acid, but no amine could subsequently be isolated.

(*c*) A solution of lithium aluminium hydride (1 g.) in ether (50 c.c.) was boiled under reflux in a Soxhlet apparatus having the dioxime (0·5 g.) in the thimble. After 5 hours' boiling, the unchanged dioxime was recovered from the solution. The experiment was repeated, and the final extraction mixture, when boiled again with an acetic acid–anhydride mixture, yielded the *diacetate* of the dioxime as yellow crystals, m. p. 192·5° (decomp.) (E.T.) after two recrystallisations from ethanol–acetone (Found: C, 61·0; H, 5·3; N, 12·9. $C_{16}H_{17}O_4N_3$ requires C, 61·0; H, 5·4; N, 13·3%).

(*d*) A solution of the dioxime (0·2 g.) in ether (35 c.c.) and 2-methoxyethanol (5 c.c.) containing palladium–carbon was unaffected when vigorously shaken with hydrogen for 2 hours at room temperature and pressure.

1 : 6-*Diketo-7-methyljulolidine.*—This was prepared similarly to (I), the reaction mixture being heated at 150° for 9 hours and, after removal of the chlorobenzene by steam-distillation, the residual mixture on slow cooling deposited the *diketone*, which when thrice recrystallised from ethanol formed bright yellow needles, m. p. 121—122° (35%) (Found: C, 72·7; H, 6·4; N, 6·6. $C_{13}H_{13}O_2N$ requires C, 72·5; H, 6·1; N, 6·5%).

When a methanolic solution of the diketone was boiled with phenylhydrazine and a few drops of acetic acid, the *bisphenylhydrazone* readily separated as fine bright yellow crystals, which when collected, washed with boiling methanol, and dried had m. p. 207—208° (97% yield) (Found: C, 75·8; H, 6·4; N, 17·7. $C_{25}H_{25}N_5$ requires C, 75·9; H, 6·4; N, 17·7%). The hydrazone was almost insoluble in all the usual solvents.

The *dioxime*, prepared similarly to that of (I), with 4·5 hours' boiling, formed pale yellow needles, m. p. 211—212° (decomp.) (E.T.) (88% yield), from aqueous ethanol (Found: C, 63·9; H, 5·9; N, 16·8. $C_{13}H_{15}O_2N_3$ requires C, 63·7; H, 6·2; N, 17·1%).

To determine the effect of a smaller proportion of aluminium chloride, a mixture of the

dinitrile (1·4 g.), aluminium chloride (4·7 g., 5 mols.), chlorobenzene (4·7 c.c.), and concentrated hydrochloric acid (0·2 c.c.) was heated at 140° for 8 hours. The cold mixture was then hydrolysed and steam-distilled as before, and the aqueous residue extracted with chloroform. Removal of the solvent left a red gum which solidified when mixed with light petroleum and was then heated at 110°/0·2 mm. to remove impurities by sublimation. The residue, when recrystallised from benzene–*cyclo*hexane, gave (in extremely low yield) pale straw-coloured crystals of a *monoketone* (presumably 1-2′-cyanoethyl-1 : 2 : 3 : 4-tetrahydro-5-methylquinol-4-one, as II), m. p. 114—115° (Found : N, 13·2. $C_{13}H_{14}ON_2$ requires N, 13·1%).

1 : 6-*Diketo*-8-*methyljulolidine*.—This *diketone* was prepared as (I), with heating of the reaction mixture at 160° for 14 hours, and after repeated recrystallisation from *cyclo*hexane was obtained as bright yellow needles, m. p. 151—152° (7% yield) (Found : C, 72·8; H, 6·2; N, 6·6. $C_{13}H_{13}O_2N$ requires C, 72·5; H, 6·1; N, 6·5%).

It formed a yellow crystalline *bisphenylhydrazone*, m. p. 269° (decomp.) (E.T.) (Found : C, 75·3; H, 6·0; N, 17·7. $C_{25}H_{25}N_5$ requires C, 75·9; H, 6·4; N, 17·7%), and a *dioxime*, pale yellow needles (from aqueous ethanol), m. p. 218° (decomp.) (E.T.) (85% yield) (Found : C, 63·7; H, 5·9; N, 17·2. $C_{13}H_{15}O_2N_3$ requires C, 63·7; H, 6·2; N, 17·1%).

8-*Hydroxy*-1 : 6-*diketojulolidine*.—This *diketone* was obtained by the usual cyclisation of bis-2-cyanoethyl-*p*-anisidine, with 15 hours' heating at 165°; it formed brilliant orange needles (from ethanol), m. p. 258—259° (decomp.) (7%) (Found : C, 66·7; H, 5·3; N, 6·5. $C_{12}H_{11}O_3N$ requires C, 66·4; H, 5·1; N, 6·45%).

It formed a bisphenylhydrazone, bright yellow crystals (from methanol), m. p. 271—272° (decomp.) (E.T.), which rapidly darkened on exposure to light.

When a solution of the diketone in acetic anhydride containing fused sodium acetate was boiled for 1 hour, cooled and poured into water, bright yellow needles of 8-*acetoxy*-1 : 6-*diketojulolidine* slowly separated from the oily deposit, and when recrystallised from ethanol had m. p. 208·5—209·5° (decomp.) (E.T.) (67% yield) (Found : C, 65·1; H, 4·9; N, 5·6. $C_{14}H_{13}O_4N$ requires C, 64·9; H, 5·05; N, 5·4%). It gave a yellow *bisphenylhydrazone*, m. p. 263° (decomp.) (E.T.) after crystallisation from ethanol (Found : C, 71·2; H, 6·05; N, 16·0. $C_{26}H_{25}O_2N_5$ requires C, 71·1; H, 5·8; N, 15·9%).

A mixture of the dinitrile (5 g.), aluminium chloride (15 g., 4·9 mols.), chlorobenzene (15 c.c.), and concentrated hydrochloric acid ( 1 c.c.) was heated at 180—190° for 5·5 hours. The mixture was hydrolysed and steam-distilled as usual, and the aqueous residue extracted with chloroform. Evaporation of the latter gave an orange-yellow solid, which after four recrystallisations from ethanol, afforded a small yield of bright yellow crystals of a *monoketone* (presumably II), m. p. 174—174·5° (E.T.) (Found : C, 67·5; H, 6·25; N, 12·0. $C_{13}H_{14}O_2N_2$ requires C, 67·8; H, 6·1; N, 12·2%).

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## 583. *Some Reactions of Sugars catalysed by a Cation-exchange Resin.*

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The cation-exchange resin, Amberlite I.R.-120, has been shown to be effective as a catalyst for the formation and hydrolysis of certain sugar glycosides and *iso*propylidene derivatives. Its catalytic activity has been compared with that of sulphuric acid in the hydrolysis of maltose, methyl-α-D-glucoside, sucrose, and starch (cf. Cadolte, Smith, and Spriestersbach, *J. Amer. Chem. Soc.*, 1952, **74**, 1501).

SUSMAN (*Ind. Eng. Chem.*, 1946, **38**, 1228) observed that acid-regenerated cation-exchange resins may sometimes advantageously replace free mineral or organic acid as catalysts for the formation of esters and for the inversion of sucrose. Bodamer and Kunin (*Amer. Chem. Soc.*, 118th Meeting, Sept., 1950) and Mariani (*Ann. Chim. appl.*, 1950, **40**, 500) studied quantitatively the rate of inversion of sucrose, and Haskell and Hammett (*J. Amer. Chem. Soc.*, 1949, **71**, 1284) studied the kinetics of hydrolysis of esters in the presence of a cation-exchange resin. They showed that reactions may be catalysed in both aqueous and non-aqueous solution, the rates being proportional to the weight of catalyst employed.

Furthermore, reduction of the particle size increased the catalytic activity, but below a certain minimum size the rate was limited by the chemical reaction rather than by the diffusion of the reactants into the body of the resin; for a maximum rate of reaction efficient agitation must be provided.

It has been observed that a number of reactions involving sugars may be satisfactorily catalysed by the solid-phase resin, providing that both the initial reactants are soluble in the liquid phase. The relative efficiency of the resin, compared with a free acid, varies with the nature of the reaction. Amberlite I.R.-120 was used for all the experiments detailed here. This resin is a strongly acidic sulphonated polystyrene of high exchange capacity, possessing excellent thermal stability, and insoluble in most solvents.

Catalysts of reactions by cation-exchange resins in place of soluble acids can greatly simplify manipulative procedure, since the catalyst may be readily removed by filtration. The removal of free acid catalysts at the end of the reaction, by means of anion-exchange resins, is sometimes impracticable since the sugars which contain potentially free aldehyde groupings may be absorbed or modified on the alkaline resin.

Hydrolyses of methyl-α-D-glucoside, maltose, and starch to glucose, inversion of sucrose, and formation of methylglucosides from glucose have been followed polarimetrically. In each case, parallel experiments, with either I.R.-120 or free sulphuric acid as catalyst, were carried out. The rate of reaction when the resin was used was, in each case, slower than when its equivalent of free sulphuric acid was used (for the determination of the equivalent weight of the resin, see the Experimental section). In contrast, Thomas and Davies (*Nature*, 1947, **159**, 372) observed enhanced catalytic activity in the hydrolysis of certain esters by sulphonic acid type cation-exchange resins in comparison with sulphuric acid.

In the presence of a cation-exchange resin, neutral salts present in the reactants may give rise to free acids by absorption of the cations. Thus, it is necessary to be cautious in attributing catalytic activity to the resin unless one can be quite certain of the absence of all anions from the reactants. In all the reactions studied here the possibility that the presence of free acid had made a significant contribution to the rate of reaction has been eliminated by checking, after the reaction was complete, that only a negligible quantity of free acid was present.

The rate of hydrolysis of a soluble starch, with a resin as catalyst, is exceedingly slow if the solution is first de-ionised by shaking it with mixed anion- and cation-exchange resins (Fig. 4, curve *B*). However, the use of a trace of free mineral acid in conjunction with the resin gives a rate of hydrolysis greater than the sum of the rates obtained when each is used separately (Fig. 4). Nevertheless, even in this case, the efficiency of the resin as a hydrolytic agent is only approximately one-twentieth of the value calculated from its equivalent weight. The addition of dry Amberlite I.R.-120 to an aqueous solution of glucose, maltose, or starch increases the optical rotation of the solution, suggesting that, while the solvent penetrates the body of the resin, the larger solute molecules do not penetrate the matrix so readily.

It therefore, appears that the inefficiency of the resin as a catalyst for the hydrolysis of starch may be due to the inability of the large molecules to penetrate the resin, but that the presence of a trace of free mineral acid degrades the starch to dextrins which may then diffuse into the body of the resin and undergo further hydrolysis by hydrogen ions associated with the resin.

A methylated starch was subjected to methanolysis and then hydrolysis in the presence of the resin, and the resultant methylated sugars obtained in high yield. The average chain length was determined by colorimetric estimation (Bartlett, Hough, and Jones, *Chem. and Ind.*, 1951, 76,) of the proportions of tetramethyl and trimethyl D-glucose and gave a value in good agreement with that obtained by hydrolysis of the same material in the presence of hydrochloric acid.

A further application of resin catalysis is in the isolation of " a reaction intermediate." Thus, when acetone is refluxed through an intimate mixture of mannitol and resin, contained in a Soxhlet thimble, a high yield of a mixture of mono- and di-*iso*propylidene mannitols was obtained, the former predominating. The reaction is largely prevented from giving

the fully substituted tri*iso*propylidene mannitol by the rapid removal, from the presence of the catalyst, of the soluble, partly substituted derivatives.

It has also been observed that the resin will catalyse the formation of methyl-α- and -β-L-arabofuranosides from L-arabinose and methyl alcohol, and of di*iso*propylidene D-glucose from glucose and acetone. 6-Methyl D-glucose has been obtained by the hydrolysis of a 6-methyl dimethylene D-glucose. A number of polysaccharides have been hydrolysed, but the possibility exists that hydrolysis was initiated by traces of acid derived from ash, present in the materials. Nevertheless, the practical utility of the method remains unimpaired, since, in each case, the amount of such free acid present was small enough to allow the solution to be evaporated to a syrup at 100° with no visible decomposition. The sugars produced on hydrolysis could then be separated and identified on the paper chromatogram in the usual manner (Partridge, *Biochem. J.*, 1947, **42**, 238).

An intimate mixture of an anion- and cation-exchange resin has been found to provide an efficient and rapid method for the removal of ash from neutral polysaccharides in both aqueous and non-aqueous solution, and no difficulty has been experienced in recovering the polysaccharides in high yield.

## Experimental

*Preparation of Resin.*—The resin used throughout these experiments was Amberlite I.R.-120, analytical grade, 40—50 mesh. The resin was washed with 0·5N-sulphuric acid (3 l. per/l. of wet resin) on a sintered-glass filter, and then with distilled water until the washings were neutral. It was then washed with alcohol, and the excess of alcohol removed under reduced pressure at 30°.

*Equivalent Weight of Resin.*—A weighed quantity of resin was shaken with a known volume of 0·1N-sodium hydroxide for 1 hour, and the excess of alkali then determined with standard acid. The equivalent weight of the resin was 344.

*Experimental Procedure.*—Reactions, unless otherwise stated, were carried out in sealed T-shaped Pyrex-glass tubes with optically flat end-plates. Agitation of the resin was accomplished by slowly rotating the tubes, which were immersed in a water-bath at the required temperature.

*Effect of Addition of Resin on Rotation of Solutions.*—An aqueous solution of glucose (5·59 c.c.; *c*, 9·95), containing a trace of ammonia, was placed in a T-shaped polarimeter tube and had α +3·19°. Dry Amberlite I.R.-120 (1·23 g.) was then added and the tube sealed and shaken for 3 hours, α was then +3·38°; this represents an absorption of 0·31 c.c. of water by the resin or 0·26 c.c. of water per g. of dry resin.

In the same manner, the resin was found to effect a change in rotation, equivalent to the absorption of 0·25 c.c. of water per/g. of dry resin from aqueous maltose, and 0·29 c.c. of water per g. of dry resin from aqueous starch.

No allowance has been made for this change in optical rotation in any of the following experiments.

*Comparative Rates of Catalysis.*—Maltose (0·273 g.), resin (1·03 g.), and water (4·5 c.c.) were sealed in the polarimeter tube and rotated in a water-bath at 100°, the optical rotation being observed at intervals. A parallel experiment was conducted with a known concentration of maltose in standard sulphuric acid. From the observed optical rotation and the known equilbrium rotations of maltose and glucose, the percentage of unhydrolysed maltose may be calculated. Fig. 1 shows the graph obtained by plotting the logarithm of the percentage of unhydrolysed maltose against time. It is linear, indicating that the reaction is of the first order. From the slope and the known concentrations of sulphuric acid and resin (with allowance for their respective equivalent weights), the resin is found to be 13% as efficient as sulphuric acid as a catalyst for this reaction.

In a similar manner, the relative efficiency of the resin as a catalyst for the hydrolysis of methyl-α-D-glucoside has been found to be 49% (Fig. 2), and in the inversion of sucrose at 25° 74% (Fig. 3).

*Hydrolysis of Starch.*—" AnalaR " soluble starch (20 g.) in water (100 c.c.) was heated at 40° for 1 hour and then de-ionised by shaking it with mixed I.R.-120–I.R.-400 resins, and all insoluble material removed on the centrifuge. The concentration of this solution (9·63% w/v) was determined by evaporating a known volume to dryness and weighing the residue. Four portions of solution were then placed in polarimeter tubes: (i) control, (ii) made N/50 with respect to sulphuric acid, (iii) containing I.R.-120 (25% w/v), and (iv) made N/50 with respect to sul-

phuric acid, I.R.-120 resin (25% w/v) being then added. Each tube was sealed and heated in a boiling-water bath. The course of the hydrolysis was followed by observing, at intervals, the change in optical rotation. The percentage hydrolysis was then calculated as for the hydrolysis of maltose. In Fig. 4 the logarithm of the percentage of starch dextrins remaining unhydrolysed is plotted against time.

*Chain-length of a Methylated Starch.*—Methylated waxy maize starch (0·3682 g.), methanol (6 c.c.), and resin (0·5 g.) were heated with shaking at 100° for 18 hours. Methanol was then removed by evaporation, and water (6 c.c.) added, and the tube resealed and heated at 100° for a further 30 hours. The resin was filtered off and the filtrate evaporated to a syrup (0·384 g.;

FIG. 1.

*A*, 0·416N *to I.R.-120 resin.*
*B*, 0·045N *to* $H_2SO_4$.

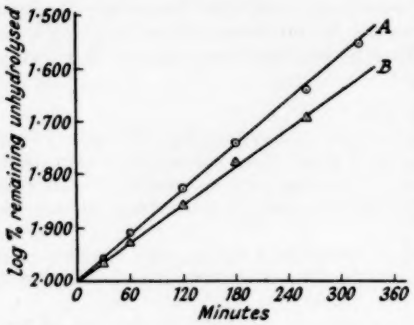



FIG. 2.

*A*, 0·312N *to I.R.-120 resin.*
*B*, 0·100N *to* $H_2SO_4$.

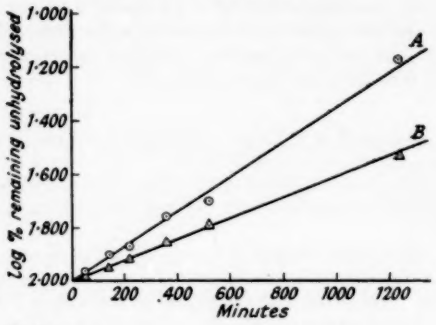



FIG. 3.

*A*, 0·46N *to I.R.-120 resin.*
*B*, 1·00N *to* $H_2SO_4$.

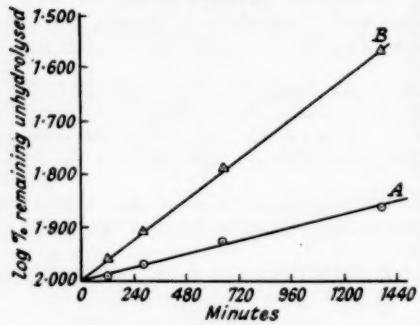
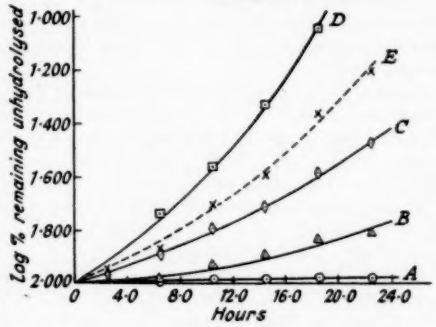



FIG. 4.

*A*, *Neutral control.*
*B*, 0·68N *to I.R.-120 resin.*
*C*, 0·02N *to* $H_2SO_4$.
*D*, 0·02N *to* $H_2SO_4$ *and* 0·88N *to I.R.-120 resin.*
*E*, *The sum of curves B and C.*




96%). End-group assay, by the colorimetric procedure, gave a chain length of 27, while a parallel experiment with hydrochloric acid gave a chain length of 25.

iso*Propylidene Mannitols.*—Mannitol (12 g.) and resin (16 g.) were intimately mixed in an extraction thimble and continuously extracted in a Soxhlet apparatus with boiling acetone for 18 hours. After cooling of the acetone extract, unchanged mannitol (0·60 g.) was removed by filtration and the filtrate evaporated to a syrup (19·43 g.), which was shaken with water (500 c.c.); crude tri*iso*propylidene mannitol was removed on the filter (4·3 g.; m. p. 68·5° on recrystallisation from light petroleum). Examination of the filtrate on the paper chromatogram, with benzene–ethanol–water (84 : 23 : 7, top layer) as the mobile phase and ammonical silver nitrate as the developer, indicated the presence of a preponderance of mono*iso*propylidenemannitol ($R_F$ 0·11), a small amount of a di*iso*propylidene derivative ($R_F$ 0·83), and a trace of mannitol ($R_F$ 0·00).

*Methylarabinosides.*—L-Arabinose (0·1 g.), methanol (2 c.c.), and resin (0·1 g.) were shaken at 20° for 24 hours. The resin and a little undissolved arabinose were removed by filtration and the filtrate was concentrated to a syrup. Examination on the paper chromatogram, with ammoniacal silver nitrate as the developer, showed the presence of methyl-α- and -β-L-arabofuranosides contaminated with small quantities of methyl-α- and β-L-arabopyranosides and -L-arabinose.

*Diisopropylidene Glucose.*—D-Glucose (0·1 g.) and resin (0·1 g.) were suspended in acetone (2 c.c.) in a sealed tube and shaken at 20° for 5 days. The resin was removed by filtration, the filtrate concentrated to a syrup, and light petroleum (5 c.c.) added. Di*iso*propylidene D-glucose crystallised, and after one recrystallisation had m. p. 109° (70 mg.).

Di*iso*propylidene D-glucose was also prepared in 60% yield by the procedure used for the *iso*propylidene mannitol derivatives.

6-*Methyl* D-*Glucose.*—A 6-methyl dimethylene D-glucose (0·1 g.), resin (0·1 g.), and methanol (2 c.c.) were heated at 100° for 18 hours. The methanol was then removed by evaporation. Water (2 c.c.) was added to the residue and the tube was resealed and heated at 100° for a further 18 hours. The resin was removed by filtration and the filtrate on concentration crystallised, to yield chromatographically pure 6-methyl D-glucose, m. p. 140°.

The author thanks Dr. J. K. N. Jones and Dr. L. Hough for their advice and encouragement, Miss J. Bartlett for carrying out the colorimetric estimations, and Mr. R. Garrard for constructing the polarimeter tubes

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## 584. *A Sexavalent Ruthenium Sulphate.*

By F. S. MARTIN.

The reduction of ruthenium tetroxide by various reducing agents in sulphuric acid has been studied. The primary reduction is thought to be from Ru(VIII) to Ru(IV) and it is shown that these two states disproportionate to form Ru(VI). Ru(VI) sulphate solutions decompose in a few hours at room temperature, probably by disproportionation to Ru(VIII) and Ru(IV). It is considered that the solution contains the complex $[RuO_2(SO_4)_2]^{--}$ rather than the ruthenyl ion $RuO_2^{++}$.

SEXAVALENT ruthenium is commonly met in the form of alkali-metal ruthenates, $M_2[RuO]_4$. These compounds are alkali stable, but unlike the analogous osmates, which yield osmyl compounds on treatment with dilute acids, the ruthenates are decomposed to salts of quadrivalent ruthenium. The ruthenyl salts are almost completely unknown and no simple salt of the type $RuO_2X_2$ has yet been isolated. Howe (*J. Amer. Chem. Soc.*, 1901, **23**, 775) treated dilute hydrochloric acid solutions containing rubidium or cæsium chloride with ruthenium tetroxide and obtained tetrachlororuthenates of the general formula $M_2[RuO_2Cl_4]$. Kraus (*Z. anorg. Chem.*, 1923, **132**, 301) obtained an ammino-derivative of sexavalent ruthenium by adding ammonia to cæsium or rubidium ruthenate. He formulated this as $RuO_2(OH)_2(NH_3)_2$ and thus it is to be regarded as a ruthenyl hydroxide derivative. The acid $H_2[RuO_2Cl_4]$ corresponding to Howe's tetrachlororuthenate was isolated by Aoyama (*ibid.*, 1924, **138**, 249) by passing hydrogen chloride and chlorine over anhydrous ruthenium tetroxide. Each of these compounds contains sexavalent ruthenium as the bivalent ruthenyl radical $RuO_2^{++}$. In the case of the tetrachlororuthenates, the reduction from the octavalent state is brought about by hydrogen chloride. The dihydroxydiammino-compound $RuO_2(OH)_2(NH_3)_2$ is derived from sexavalent ruthenium by a process involving no valency changes, *i.e.*,

$$M_2[RuO_4] + NH_4{\cdot}OH \longrightarrow \underset{\text{(possible intermediate)}}{(NH_4)_2[RuO_4]} \longrightarrow RuO_2(OH)_2(NH_3)_2.$$

Rémy and Lührs (*Ber.*, 1928, **61**, 917) observed that the reduction of Ru(VIII) by hydrogen chloride does not go to Ru(VI) in one step, but rather that the primary reduction is to Ru(IV) which reacts with excess of ruthenium tetroxide to form Ru(VI). More recently,

Wehner and Hindman (*J. Amer. Chem. Soc.*, 1950, **72**, 3911) investigated the electrolytic reduction of Ru(VIII) in perchloric acid solution but found no evidence for the existence of a sexavalent state in this acid.

This paper describes the reduction of Ru(VIII) in dilute sulphuric acid solutions by means of sodium nitrite, sodium sulphite, ferrous sulphate, hydrazine, and hydroxylamine. It is shown that a sexavalent state of ruthenium is capable of existence in sulphuric acid solution. The presence of this valency state can only be due to the ruthenyl radical. Ruthenates are immediately unstable in acid solution, and in addition, the ruthenate ion has a pronounced orange colour in solution whereas the sexavalent ruthenium solution described here is green. Quadrivalent ruthenium sulphate solutions are intensely brown.

## Experimental

Known amounts of reducing agent were added to an excess of ruthenium tetroxide dissolved in sulphuric acid. Reduction occurred immediately except in the case of hydrazine where there was frequently a variable induction period. The excess of ruthenium tetroxide was extracted from the solution by carbon tetrachloride, and analysis for ruthenium then gave directly the quantity of Ru(VIII) which had been reduced.

Results are presented in the table. The consumption of reducing agent per mole of Ru(VIII) is clearly dependent on the relative excess of Ru(VIII). With a ruthenium tetroxide concentration greater than about six-fold the amount of reducing agent, the consumption of the latter

*Consumption of reducing agent per mole of* $RuO_4$ *in sulphuric acid solution.*

| Micro-moles of reducing agent | Micro-moles of $RuO_4$ reduced | Equiv. of reducing agent per mole of $RuO_4$ | Original excess of $RuO_4$ (approx.) | Colour of product | Valency of Ru in product |
|---|---|---|---|---|---|
| $NaNO_2$ | | | | | |
| 13·4 | 13·6 | 2·0 | 6-fold | Green | 6 |
| 13·4 | 13·1 | 2·0 | 5 ,, | Green | 6 |
| 67·0 | 48·5 | 2·8 | 4 ,, | Green-brown | Mixed 4 and 6 |
| 67·0 | 39·8 | 3·4 | 3 ,, | ,, | ,, ,, |
| 67·0 | 38·8 | 3·5 | 2 ,, | ,, | ,, ,, |
| 67·0 | 36·8 | 3·7 | 1 ,, | ,, | ,, ,, |
| $Na_2SO_3$ | | | | | |
| 15·4 | 15·4 | 2·0 | 7-fold | Green | 6 |
| 15·4 | 14·8 | 2·1 | 5 ,, | Green | Mainly 6 |
| 15·4 | 12·6 | 2·5 | 4 ,, | Green-brown | Mixed 4 and 6 |
| 58·0 | 30·6 | 3·9 | <1 ,, | Green-brown | ,, ,, |
| 58·0 | 29·0 | 4·0 | nil | Brown | 4 |
| $N_2H_4,H_2SO_4$ | | | | | |
| 22·2 | 33·0 | 2·7 | 4-fold | Green-brown | Mixed 4 and 6 |
| 22·2 | 28·7 | 3·1 | 3 ,, | ,, | ,, ,, |
| 44·3 | 51·2 | 3·5 | 2 ,, | ,, | ,, ,, |
| $2NH_2{\cdot}OH,H_2SO_4$ | | | | | |
| 8·1 | 16·2 | 2·0 | 8-fold | Green | 6 |
| 8·1 | 13·5 | 2·4 | 4 ,, | Green-brown | Mixed 4 and 6 |
| 8·1 | 13·0 | 2·5 | 3·5-fold | ,, | ,, ,, |
| $FeSO_4$ | | | | | |
| 9·4 | 4·8 | 2·0 | 8-fold | Green | 6 |
| 9·4 | 4·6 | 2·0 | 6 ,, | Green | 6 |

is 2 equiv. per ruthenium atom, the product evidently being Ru(VI). With smaller relative amounts of Ru(VIII), reduction involves the consumption of more than 2 equiv. per ruthenium atom, the maximum consumption of reducing agent being 4 equiv. The product is then Ru(IV), and for the intermediate cases must be a mixture of Ru(IV) and Ru(VI). The colour of the Ru(VI) solution is green, and that of Ru(IV) the familiar dark brown. The colour of the mixed solutions is intermediate.

The valency of 6 for the ruthenium in the green solution was verified [after extraction of unchanged Ru(VIII) by carbon tetrachloride] by Crowell and Yost's method (*J. Amer. Chem. Soc.*, 1928, **50**, 374) in which the ruthenium is reduced to the +3 state by iodide ion, and the liberated iodine estimated volumetrically.

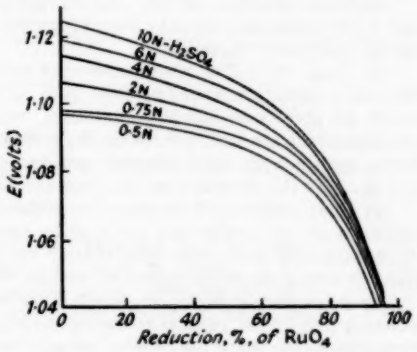
The potentiometric titration of Ru(VIII) in dilute sulphuric acid with any of the reducing

agents cited above, by using a bright platinum electrode and saturated calomel reference electrode, merely showed steps in the titration curves corresponding to the consumption of 4 equiv. of reducing agent per mole of Ru(VIII). No steps were observed corresponding to the reduction to Ru(VI), although the green colour of this valency state was observed in the earlier parts of the titration.

The redox potential of the system when titrated with ferrous ion was dependent on the sulphuric acid concentration. The figure shows that the dependence is roughly of the order associated with hydrogen-ion consumption in reductions of the type $RuO_4 + 8H^+ + 4e^- \longrightarrow Ru^{4+} + 4H_2O$ and $RuO_2^{++} + 4H^+ + 2e^- \longrightarrow Ru^{4+} + 2H_2O$, where the value of the redox potential is given approximately by

$$E_{Ru(VIII)/Ru(IV)} = E_{0Ru(VIII)/Ru(IV)} - \frac{RT}{4F} \ln \frac{[Ru^{4+}]}{[RuO_4][H^+]^8}$$

At the points on the curves corresponding to 50% reduction, the solution contains a mixture of Ru(VIII), Ru(VI), and Ru(IV), and the observed potential at these points may therefore be due to both the Ru(VIII)/Ru(IV) and the Ru(VI)/Ru(IV) couple. Since no step is observed in the potential curves corresponding to the reduction of Ru(VIII) to Ru(VI), and also since both Ru(VI) and Ru(IV) may be present in the partially reduced solution, the primary reduction may possibly be directly to Ru(IV), with subsequent formation of Ru(VI) by interaction with excess of Ru(VIII). That this interaction is possible was confirmed by taking freshly reduced Ru(IV) solution in




*Dependence of redox potential of* Ru(VIII) *system on* $H_2SO_4$ *concentration.* [Ru(VIII)] = $10^{-2}$M.

dilute sulphuric acid and adding to it an excess of Ru(VIII). The green colour of Ru(VI) developed immediately, and after removal of excess of Ru(VIII), the sexavalency was confirmed by Crowell and Yost's method (*loc. cit.*). Solutions of Ru(IV) more than a few hours old, or solutions which have been heated, do not lead to the formation of Ru(VI) on addition of the tetroxide. The quadrivalent ruthenium ion is not stable in the simple form in solution, and its failure to disproportionate with Ru(VIII) when not freshly prepared is probably connected with the formation of either a more stable hydrolysis product, or a sulphate complex on storage, or possibly even oxygen-bridge formation.

Ru(VI) solutions in dilute sulphuric acid decompose to Ru(IV) in a few hours at room temperature, even when excess of Ru(VIII) is present. Since it is formed by reproportionation of Ru(IV) and Ru(VIII), it would be reasonable to expect it to decompose by disproportionation to these two states. The presence of Ru(VIII) was not unambiguously shown in the decomposition products, but its transient concentration would be sufficiently low for a large amount of reduction to occur on glass surfaces or adventitious dust particles. Higher concentrations cannot be used to obviate this, since the maximum concentration of ruthenium tetroxide in dilute sulphuric acid is of the order of 2%, and as shown in the table, it is not possible to prepare from this a solution of Ru(VI) of greater concentration than about one-sixth of 2%, corresponding approximately to $2 \times 10^{-2}$M. However, if the disproportionation occurs according to $2Ru(VI) \longrightarrow RuO_4 +$ Ru(IV), then the iodine-liberating power from iodide ion should be the same for both products and reactant, and the iodine titre of the mixture should not vary with time except in so far as Ru(VIII) is decomposed by surface reactions as mentioned above. On the other hand, the possible alternative method of decomposition is $RuO_2^{++} + 2H^+ \longrightarrow Ru^{4+} + H_2O + \frac{1}{2}O_2$, and the iodine titre in this case should fall to one-third of its initial value when decomposition is com-

plete. In practice, the iodine titre–time curves were not reproducible, but decreases of not more than 10% were observed in the titres, and this clearly obviates the alternative mode of decomposition. Additional evidence in favour of decomposition by disproportionation is obtained from the study of the potential at a platinum electrode in a solution of Ru(VI) in sulphuric acid. A saturated calomel reference electrode being used, the potentials for freshly prepared Ru(VI) solutions were of the order of 0·8 v, rising steadily for a period of about 15 minutes to a maximum value of the order of 1·0—1·1 v. As may be seen from the figure, this latter value is that associated with a solution containing both Ru(VIII) and Ru(IV) as well as Ru(VI), and it is concluded that the presence of Ru(VIII) and Ru(IV) has arisen by disproportionation of Ru(VI).

(a) *Preparation of Reagents.*—Ruthenium tetroxide was prepared by fusing ruthenium sponge or ruthenium trichloride with potassium nitrate and potassium hydroxide. If the sponge was used the melt containing potassium ruthenate was dissolved in water and acidified with sulphuric acid, and sufficient periodic acid was added to oxidise the ruthenate to Ru(VIII). In the case of the trichloride, the melt was dissolved, and hydrated Ru(IV) oxide precipitated by addition of methanol. The precipitate was filtered off, washed, dried, and re-fused. The second melt was then treated as for the sponge. After addition of the periodic acid, the ruthenium tetroxide was distilled off in a current of air with gentle heating, and was condensed into ice-cold dilute sulphuric acid. The tetroxide was redistilled from this solution and was collected as the yellow solid (m. p. 25°) on an ice-cold surface. The solid was allowed to melt and was dissolved in dilute reagent-grade sulphuric acid.

Solutions of sodium nitrite, sodium sulphite, ferrous ammonium sulphate, hydrazine sulphate, and hydroxylamine sulphate were prepared from pure or A.R. reagents and were standardised by the customary methods.

(b) *Analysis.*—The determination of ruthenium was carried out according to Marshall and Rickard's absorptiometric method (U.S.A.E.C. unclassified document number AECU 224) in which an aliquot of solution containing ruthenium is evaporated and the residue fused with potassium nitrate and potassium hydroxide. The melt is dissolved to a known volume in 2N-potassium hydroxide, and the orange ruthenate colour is measured by the absorbancy at 465 mμ. In the present case the determination was carried out by " Spekker " absorptiometer.

(c) *Confirmation of Valency.*—Crowell and Yost (*loc. cit.*) have shown that higher ruthenium valencies are reduced to the +3 state by potassium iodide. Aliquots of the ruthenium solution in 2N-sulphuric acid were treated with *ca.* 1 g. of potassium iodide, and the liberated iodine titrated with sodium thiosulphate, carbon tetrachloride being used as " indicator."

(d) *Reduction of* $RuO_4$ *in Dilute Sulphuric Acid.*—Aliquots of $RuO_4$–sulphuric acid were pipetted into 15-ml. conical centrifuge tubes held in an ice-bath. Aliquots of reducing solution were added (the tip of the pipette being below the surface of the solution for $NaNO_2$ and $Na_2SO_3$ solutions) and the contents were stirred for a few seconds. Reaction was immediate except in the case of hydrazine, when there was often an induction period of several minutes. An approximately equal volume of carbon tetrachloride was added, and the two phases mixed by stirring. After being allowed to settle, the organic phase was removed, and the process was repeated twice more. The partition coefficient of ruthenium tetroxide between carbon tetrachloride and water is 59 (unpublished results) in favour of the organic phase and therefore three equal-volume washes reduce the tetroxide concentration to negligible proportions. After removal of the last droplets of solvent by centrifuging the tubes for a few minutes, aliquots of solution were withdrawn for ruthenium analysis.

## Discussion

Since the formation of Ru(VI) in sulphuric acid solution may occur by reproportionation of Ru(VIII) and Ru(IV), it is logical to expect the decomposition of Ru(VI) by disproportionation and the equilibrium $2Ru(VI) \rightleftharpoons Ru(VIII) + Ru(IV)$ to be set up. Approximate values may be obtained from the table and substituted in the expression $[Ru(VIII)][Ru(IV)]/[Ru(VI)]^2$ and a set of evaluations lying mainly between 20 and 80 is obtained. Hence, if this expression represents the equilibrium constant for the disproportionation reaction, disproportionation should be 20—80 times as rapid as reproportionation. Since reproportionation has been shown to be immediate [where freshly prepared Ru(IV) is concerned], the solution of Ru(VI) when separated from Ru(VIII) should immediately disproportionate to the extent of approximately 90%. In fact the disproportionation to this extent takes at least 1—2 hours. It is evident then that $2RuO_2^{++} \rightleftharpoons RuO_4 + Ru^{4+}$ does not represent the true approach to equilibrium, and not only must complex species of Ru(IV) be taken into

account, but also of Ru(VI). It would be expected that the Ru(VI) would not exist as the simple or hydrated $RuO_2^{++}$ ion in sulphuric acid solution, but more probably as $[RuO_2(SO_4)_2]^{--}$ (cf. the tetrachlororuthenates). Transference experiments, however, failed to reveal the presence of anionic Ru(VI) in solution, but it is believed that this may be due to the rapid decomposition of Ru(VI) on passing into a region of zero Ru(VI) concentration, *i.e.*, into the anode or cathode compartments of the transference cell. Evidence in favour of this belief was obtained when diluting Ru(VI) solutions for spectrophotometric examination. On dilution to a ruthenium concentration of approximately $10^{-4}$M and maintaining the sulphuric acid concentration at 2N, decomposition of Ru(VI) to Ru(IV) was complete in 30 minutes as compared with periods of more than 2 hours at concentrations of $10^{-2}$M. The decomposition was too rapid at the lower concentrations to enable spectrophotometric data to be obtained.

*Conclusions.*—It has been shown that solutions of sexavalent ruthenium involving the ruthenyl radical $RuO_2^{++}$ may be prepared in sulphuric acid solutions by reduction of ruthenium tetroxide. The ruthenyl compound may be formed by way of reproportionation of Ru(VIII) and Ru(IV) and it probably decomposes by the corresponding disproportionation reaction. Qualitative observation of the rates of reproportionation and disproportionation indicates that it is unlikely that the simple or hydrated $RuO_2^{++}$ ion exists in solution.

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## 585. *Trypanocides of the Phenanthridine Series. Part I. The Effect of Changing the Quaternary Grouping in Dimidium Bromide.*

By T. I. WATKINS.

It has been found that a change of quaternary grouping in 2 : 7-diamino-10-methyl-9-phenylphenanthridinium bromide ("Dimidium bromide") has a marked effect on the curative power of the drug in *Trypanosoma congolense* infection of mice and cattle. Eight quaternary analogues have been prepared.

2 : 7-DIAMINO-10-METHYL-9-PHENYLPHENANTHRIDINIUM BROMIDE ("Dimidium bromide") (I; R = Me, A = Br) (Walls, *J.*, 1945, 294) shows high activity against *Trypanosoma congolense* in laboratory tests in mice and in the field in cattle. Although analogues containing a variety of substituents in different positions have been prepared, particularly by Walls (*J.*, 1938, 389, and subsequent papers), "Dimidium bromide" is still the drug of choice, within this group of compounds, for treatment of bovine trypanosomiasis. A quaternary group in the molecule is essential for activity as the unquaternised compound is inactive. Quaternisation of the ring nitrogen atom markedly increases its basicity and it might therefore be expected that interference with the basic strength of this nitrogen, by variation of the nature of the quaternary group, would lead to changes in the activity of the molecule. In fact, substitution of the methyl by other quaternary groupings was found to cause marked changes in the curative power of the drug. The compounds prepared [I; R = Et, A = Br; R = $Pr^n$, A = Br; R = $Bu^n$, A = Cl; R = *n*-amyl, A = Cl; R = *n*-hexyl, A = Cl; R = allyl, A = Br; R = $[CH_2]_3{\cdot}NHEt_2\}I$, A = I; R = $[CH_2]_3{\cdot}NEt_2Me\}I$, A = I] were tested against *Trypanosoma congolense* in mice and three of these compounds (I; R = Et, A = Br; R = $Pr^n$, A = Br; R = allyl, A = Br) were outstandingly active, being many times more active (and less toxic) than dimidium bromide. The pharmacological results obtained with mice and also the results of preliminary field trials with cattle in Africa have been published elsewhere (Watkins and Woolfe, *Nature*, 1952, **169**, 506; Woolfe, *Ann. Trop. Med. Parasit.*, in the press)].

The greatly increased activity of some of the compounds is probably a result of variation of the basic strength of the ring nitrogen atom, since introduction of alkyl groupings at

other positions has little effect (unpublished work). The changes in the basic strength of the ring nitrogen atom can, however, only be small and, since, in each case, dissociation of the drugs in aqueous solution at neutral pH's is nearly complete (>99·8%), the changes effected in concentration of cations will be insignificant and certainly not great enough to account for the magnitude of the enhancement in activity. Physical measurements (by R. Hughes) on aqueous solutions of the compounds at physiological pH's, however, indicate that there are marked changes in the small concentration of drug present in the pseudo-base form. Thus a change of methyl to ethyl quaternary grouping results in a 2- or 3-fold increase in concentration of the pseudo-base form at pH 6—9. An increased concentration of neutral component (either of the pseudo-base or ion-pair) may be responsible for an increased rate of diffusion across the cell membrane into the trypanosome cytoplasm (cf. Davson and Danielli, " Permeability of Natural Membranes," Cambridge Univ. Press 1943).

Some of the compounds had a prophylactic effect in mice considerably greater than that of dimidium bromide. One such, 10-allyl-2 : 7-diamino-9-phenylphenanthridinium bromide (I; R = allyl, A = Br), was examined further with the view to increasing its prophylactic power. A change of anion to give the more insoluble iodide, perchlorate, and 2-chloro-3 : 5-dinitrobenzoate did not extend the prophylactic period appreciably. Similarly, substitution in the 2- and the 7-amino-group to form bis(sodium formaldehyde bisulphite), diglucoside, and bis(tetra-acetyl glucosyl) derivatives, had little effect in increasing the prophylactic power.

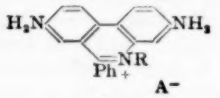
The yields of quaternary compounds obtained were low compared with that of the methyl compound, owing to the marked progressive decrease in rate of reaction of the alkylating agents as the length of the alkyl chain increased (cf. Preston and Jones, *J.*, 1912, **101**, 1931). This paralleled a decreasing stability of the reagents at the temperatures necessary for reaction.

The alkyl quaternary compounds were prepared from 2 : 7-biscarbethoxyamino-9-phenylphenanthridine and the appropriate toluene-*p*-sulphonic ester. The conditions for reaction were, as far as possible, so chosen that minimum decomposition of the toluene-*p*-sulphonic ester occurred. In every reaction, however, there was a partition of the phenanthridine between (*a*) the quaternisation reaction and (*b*) its fixation as a salt with the toluene-*p*-sulphonic acid formed by decomposition of the ester.

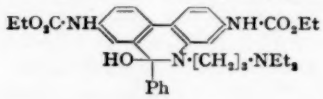
When attempts were made to prepare quaternary compounds with branched alkyl chains, the decomposition of the esters was much the faster reaction and in no case was the pure quaternary compound obtained. This was not surprising as the rate of quaternisation in these cases would be greatly decreased by steric hindrance.

In the preparation of the allyl quaternary compound, allyl iodide was used, as allyl toluene-*p*-sulphonate is known to decompose violently when heated (Gilman and Beaber, *J. Amer. Chem. Soc.*, 1925, **47**, 522).

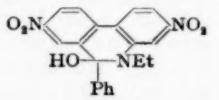
The 3-bromopropyl quaternary salt prepared from 2 : 7-biscarbethoxyamino-9-phenylphenanthridine and 1 : 3-dibromopropane, when treated with diethylamine, afforded a diethylaminopropyl quaternary compound but this was isolated from the alkaline solution as the pseudo-base (II), which was then converted into the quaternary chloride hydrochloride. Further, the diethylamino-group could itself be quaternised. This reaction was



(I)



(II)



(III)

carried out with the pseudo-base, since attempts to liberate the diethylamino-group from the hydrochloride with alkali simultaneously converted the quaternary salt into the pseudo-base. From the reaction between the pseudo-base and methyl sulphate, 2 : 7-biscarbethoxyamino-10-3′-diethylaminopropyl-9-phenylphenanthridinium hydrogen sulphate 3′-methomethylsulphate was obtained. Hydrolysis of the pseudo-base (II) (or a corresponding salt) and its quaternary derivative proceeded normally in hot sulphuric acid solution.

The ethyl quaternary compound (I; R = Et, A = Br), which has been named " Ethidium bromide," has also been prepared from 2 : 7-dinitro-9-phenylphenanthridine by using excess of ethyl toluene-*p*-sulphonate as solvent. The effects of time and temperature on this reaction were critical as an apparent autocatalytic decomposition of the quaternary product set in after the attainment of an optimum yield and, on prolonged heating, extensive charring occurred. In the early stage of the reaction the unchanged phenanthridine exerts a " buffering " action in that it removes by salt formation the free toluene-*p*-sulphonic acid formed during decomposition of the ester. A point is reached in the reaction where no unchanged phenanthridine is present. The free toluene-*p*-sulphonic acid then initiates autocatalytic decomposition of the ester which may in turn be responsible for the observed increase in rate of decomposition of the quaternary product. For preparative purposes, therefore, it was found best to stop the reaction while there was still some unchanged dinitrophenanthridine present. This was recovered and the quaternised material was then reduced and isolation of the required material was facilitated by the ease with which the ethyl ether of its pseudo-base could be extracted into toluene. Re-extraction into acid converted the ether into a quaternary salt.

In studying the conditions for the quaternisation reaction the quaternary product was isolated as the pseudo-base (III).

## Experimental

*2 : 7-Biscarbethoxyamino-9-phenylphenanthridine.*—This compound is described by Walls (*J.*, 1947, 67). It was found that a purer product was more readily obtained by reduction of 2 : 7-dinitro-9-phenylphenanthridine and subsequent carbethoxylation. Powdered 2 : 7-dinitro-9-phenylphenanthridine (14·3 g.) (Walls, *J.*, 1945, 294) and iron powder (17 g.) were stirred together in water (300 c.c.), alcohol (50 c.c.), and 5N-hydrochloric acid (2 c.c.), and the mixture was heated under reflux for 5 hours. The whole was then cooled, made alkaline with ammonia solution, and filtered, and the residue washed with water. 2 : 7-Diamino-9-phenylphenanthridine was extracted from the dried solid residue with chloroform and, on concentration of the extract, separated in yellow prisms, m. p. 197—198° (10·75 g., 91%) (Walls, *loc. cit.*, records m. p. 198°). A stirred solution of the diamine (10·4 g.) in dry pyridine (75 c.c.) was treated with ethyl chloroformate (7·5 c.c.) at 25—30°. The dark red solution was slowly stirred at room temperature for 5 hours, then poured into water, and the yellow solid collected. It crystallised from aqueous methanol as white needles, m. p. 138° (not sharp) (Found: C, 70·3; H, 5·2; N, 10·0. Calc. for $C_{25}H_{23}O_4N_3$: C, 69·9; H, 5·4; N, 9·8%). This compound, which is not described by Walls, was also obtained by his procedure. Heating with water for 2 hours converted it into Walls's high-melting compound, m. p. 218—220° (decomp.) (12·5 g., 80%).

*Toluene-*p*-sulphonic Esters.*—The following esters were prepared by the general method of Marvel and Sekera (*Org. Synth.*, 1940, **20**, 50): ethyl, *n*-propyl, *iso*propyl, *n*-butyl, *iso*butyl, *n*-amyl, *iso*amyl, and *n*-hexyl. Each was repeatedly fractionated to remove traces of toluene-*p*-sulphonyl chloride. Decomposition of the esters was catalysed by small amounts of the sulphonyl chloride during the quaternisation reactions, with consequent loss in yield of quaternary product.

*General Method of Preparation of the Quaternary Compounds.*—2 : 7-Biscarbethoxyamino-9-phenylphenanthridine and the sulphonic ester (2 mols.) were heated with slow stirring either alone or in nitrobenzene under various conditions of time and temperature. On cooling, the crude quaternary salt separated or was precipitated by dilution with ether. It was hydrolysed by heating it with 75—80% (w/w) sulphuric acid at 125—130° according to Walls's general method (*J.*, 1947, 67). The cooled mixture was added to water or, in the case of the higher alkyl compounds, to aqueous alcohol, and the pH was raised to 7—7·5 by dilute ammonia solution. The mixture was then heated and the pH again adjusted to 7—7·5 if necessary. Ammonium bromide (or ammonium chloride when the final product was required as a chloride) was added, and the precipitated tar was extracted with hot water. Ammonium bromide (or chloride) was again added to the filtered extract to precipitate the crude quaternary halide. Crystallisation from an alcoholic solvent yielded the pure quaternary salt.

In the reaction of 2 : 7-biscarbethoxyamino-9-phenylphenanthridine with *iso*propyl, *iso*butyl, and *iso*amyl toluene-*p*-sulphonates under the above conditions, no crystalline quaternary compound was isolated. There was much evolution of gas (olefin) throughout each experiment, even at relatively low temperatures, and the major product was 2 : 7-biscarbethoxyamino-9-

phenylphenanthridine toluene-*p*-sulphonate. If the hydrolysis with sulphuric acid was omitted, an 85—90% yield of recovered 2 : 7-biscarbethoxyamino-9-phenylphenanthridine was obtained by basification.

2 : 7-*Diamino*-10-*ethyl*-9-*phenylphenanthridinium Bromide*.—Quaternisation was effected in nitrobenzene solution during 2·5 hours at 155—160°. The *bromide* crystallised from methanol as dark red elongated plates, m. p. 248—249° (decomp.) (55% from 2 : 7-biscarbethoxyamino-9-phenylphenanthridine). Even after 10 hours' drying at 100° it retained 1·2% of water (Found : C, 62·8; H, 5·1; N, 10·3; Br, 19·85. $C_{21}H_{20}N_3Br$ containing 1·2% of water requires C, 63·2; H, 5·2; N, 10·5; Br, 20·05%. Found, for compound dried for 2 hours at 150°/1 mm. : N, 10·7; Br, 20·1; $H_2O$, 0·1. $C_{21}H_{20}N_3Br$ requires N, 10·65; Br, 20·3%). The *ethonaphthalene*-β-*sulphonate monohydrate* crystallised from methanol–propan-2-ol as dark red plates, m. p. 255° (decomp.) (Found : N, 7·7; $H_2O$, 3·7. $C_{31}H_{29}O_4N_3S$ requires N, 7·8; $H_2O$, 3·3%). (For the alternative method of preparation of this compound from 2 : 7-dinitro-9-phenylphenanthridine, see below.)

2 : 7-*Diamino*-9-*phenyl*-10-n-*propylphenanthridinium Bromide*.—Quaternisation was effected in nitrobenzene during 6 hours at 155—160°. The *bromide* was obtained in 25% yield as red plates (from methanol–propan-2-ol), m. p. 261—263° (decomp.) (Found : C, 64·6; H, 5·7; N, 10·35; Br, 19·0. $C_{22}H_{22}N_3Br$ requires C, 64·7; H, 5·4; N, 10·3; Br, 19·6%). The intermediate 2 : 7-*biscarbethoxyamino*-9-*phenyl*-10-n-*propylphenanthridinium toluene*-p-*sulphonate* crystallised from propan-2-ol or a large volume of water as orange needles, m. p. 220—222° (decomp.; slow heating) (Found : N, 6·7. $C_{35}H_{37}O_7N_3S$ requires N, 6·5%).

2 : 7-*Diamino*-10-n-*butyl*-9-*phenylphenanthridinium Chloride*.—Quaternisation was effected in nitrobenzene solution during 4 hours at 150°. The decomposition of ester became rapid after this time. The *chloride* was obtained in 22% yield as purple plates (from propan-2-ol), m. p. 238—240° (decomp.) (Found, in compound dried for 4 hours at 100° : N, 10·5; Cl, 9·6; $H_2O$, 1·7. $C_{23}H_{24}N_3Cl,0·5H_2O$ requires N, 10·8; Cl, 9·2; $H_2O$, 2·3. Found, in compound dried for 2 hours at 150°/1 mm. : N, 11·0; Cl, 9·2; $H_2O$, 0. $C_{23}H_{24}N_3Cl$ requires N, 11·1; Cl, 9·4%).

2 : 7-*Diamino*-10-n-*amyl*-9-*phenylphenanthridinium Chloride*.—Quaternisation was effected without a solvent during 4 hours at 150°. The *chloride* was obtained in 24% yield as purple plates (from propan-2-ol), m. p. 235—238° (decomp.; slow heating) (Found : N, 10·9; Cl, 8·7. $C_{24}H_{26}N_3Cl$ requires N, 10·7; Cl, 9·1%). Before hydrolysis partial purification of the intermediate 10-*n*-amyl-2 : 7-biscarbethoxyamino-9-phenylphenanthridinium toluene-*p*-sulphonate was effected by extraction of the crude product with warm methanol and precipitation with ether.

2 : 7-*Diamino*-10-n-*hexyl*-9-*phenylphenanthridinium Chloride*.—Quaternisation (without solvent; 6 hours; 150°) gave the *chloride* in 22% yield as purple plates (from propan-2-ol), m. p. 240—241° (decomp.; slow heating) (Found : N, 10·5; Cl, 8·4. $C_{25}H_{28}N_3Cl$ requires N, 10·3; Cl, 8·7%). Partial purification of the intermediate 2 : 7-biscarbethoxyamino-10-*n*-hexyl-9-phenylphenanthridinium toluene-*p*-sulphonate was effected as for the *n*-amyl compound.

10-*Allyl*-2 : 7-*diamino*-9-*phenylphenanthridinium Bromide*.—2 : 7-Biscarbethoxyamino-9-phenylphenanthridine and allyl iodide (2·5 mols.) were heated together with slow stirring in nitrobenzene at 95—100° for 4 hours. 10-*Allyl*-2 : 7-*biscarbethoxyamino*-9-*phenylphenanthridinium iodide*, which separated as orange needles during the heating, was collected after cooling of the mixture (66%). A specimen crystallised from ethanol–ether as orange needles, m. p. 220° (decomp.; slow heating) (Found : N, 7·0; I, 21·5. $C_{28}H_{28}O_4N_3I$ requires N, 7·0; I, 21·3%). To avoid complications arising from the liberation of iodine and hydrogen iodide by action of sulphuric acid during the hydrolysis, the crude iodide was converted in 92% yield into the corresponding methanesulphonate by silver methanesulphonate in aqueous methanol which had been acidified with methanesulphonic acid. It separated as yellow needles, m. p. *ca.* 220° (decomp.; slow heating). The methanesulphonate was then hydrolysed with 75—80% (w/w) sulphuric acid, and the product finally isolated as a quaternary *bromide* (49%), dark red (from water) or permanganate-coloured plates (from ethanol), m. p. 230—231° (decomp.) (Found : C, 64·85; H, 5·1; N, 10·35; Br, 19·2. $C_{22}H_{20}N_3Br$ requires C, 65·2; H, 5·0; N, 10·3; Br, 19·7%). 10-*Allyl*-2 : 7-*diamino*-9-*phenylphenanthridinium iodide*, prepared from the bromide, crystallised from water as small red needles, m. p. 234—235° (decomp.) (Found : N, 9·5; I, 27·6. $C_{22}H_{20}N_3I$ requires N, 9·3; I, 28·0%). 10-*Allyl*-2 : 7-*diamino*-9-*phenylphenanthridinium perchlorate*, prepared from the bromide, crystallised from hot water containing a little potassium perchlorate as small red needles, m. p. 250—252° (decomp.) (Found : N, 10·0. $C_{22}H_{20}O_4N_3Cl$ requires N, 9·8%). 10-*Allyl*-2 : 7-*diamino*-9-*phenylphenanthridinium* 2-*chloro*-3 : 5-*dinitrobenzoate*, prepared from the bromide, crystallised from ethanol as a microcrystalline red solid, m. p. 243—245° (decomp.) (Found : N, 12·5. $C_{29}H_{22}O_6N_5Cl$ requires N, 12·3%).

10-*Allyl*-2 : 7-*diamino*-9-*phenylphenanthridinium Iodide Bis*(*formaldehyde Bisulphite*) *Dihydrate*.—10-Allyl-2 : 7-diamino-9-phenylphenanthridinium bromide, sodium formaldehyde bisulphite (2·2 mols.), and sufficient water to form a solution at 100° were heated at this temperature for 2 hours. The solution was decanted from a small amount of tar, potassium iodide (*ca.* 4 mols.) added, and ethanol then added to the hot solution until crystallisation commenced. The purple *salt* obtained on cooling crystallised from aqueous ethanol as small purple plates, m. p. >310° (72%) (Found : N, 5·6; $H_2O$, 4·5. $C_{24}H_{22}O_6N_2S_2INa_2,2H_2O$ requires N, 5·8; $H_2O$, 5·0%).

10-*Allyl*-2 : 7-*bisglucosylamino*-9-*phenylphenanthridinium Bromide*.—10-Allyl-2 : 7-diamino-9-phenylphenanthridinium bromide (4 g.), glucose monohydrate (4·1 g., 2·1 mols.), acetic acid (1·2 c.c., 2 mols.), methanol (80 c.c.), and ammonium chloride (trace) were boiled for 3 minutes and the solution then kept at 40° for 5 hours. Ether was added to precipitate a red solid which was separated and extracted with cold water (30 c.c.). The filtered aqueous extract was evaporated to dryness in a vacuum and the residue crystallised from ethanol–ether as a microcrystalline red solid (70%), m. p. *ca.* 200° (with charring) (Found : N, 5·7. $C_{24}H_{40}O_{10}N_2Br$ requires N, 5·8%).

10-*Allyl*-9-*phenyl*-2 : 7-*bis*(*tetra-acetyl glucosylamino*)*phenanthridinium Bromide*.—The above glucoside in pyridine solution was slowly treated at 25° with acetic anhydride (11 mols.), and the solution then set aside overnight. Addition of ether precipitated a pink solid, and water was then added to the mixture until the solid dissolved. The aqueous layer was separated and extracted several times with ether until it deposited an orange-coloured solid, which was collected, washed with ice-water, and dried in a vacuum. It crystallised from ethanol–ether as a microcrystalline pink solid (78%), m. p. 185° (with charring) (Found : N, 4·3; Br, 8·3. $C_{50}H_{56}O_{18}N_2Br$ requires N, 3·9; Br, 7·5%).

10-3′-*Bromopropyl*-2 : 7-*biscarbethoxyamino*-9-*phenylphenanthridinium Bromide*.—2 : 7-Biscarbethoxyamino-9-phenylphenanthridine (22 g.) was heated with stirring with 1 : 3-dibromopropane (60 c.c.) in an oil-bath at 170—175° for 60 minutes. Yellow plates were deposited after 20 minutes. The mixture was cooled, and dry ether (40 c.c.) was added, and the mixture filtered. The yellow solid was washed with dry ether and dried at 100° [yield 30 g.; m. p. 195—200° (decomp.)]. Recrystallisation of the *bromide* from a large volume of ethanol yielded yellow plates, m. p. 235—236° (decomp.) (27·5 g., 84%) (Found : C, 52·7; H, 5·0; N, 6·5; Br, 23·8. $C_{28}H_{29}O_4N_3Br_2$ requires C, 53·3; H, 4·6; N, 6·7; Br, 25·4%).

2 : 7-*Biscarbethoxyamino*-10-3′-*diethylaminopropyl*-9-*phenylphenanthridinium Chloride Hydrochloride*.—The above bromopropyl quaternary compound (20 g.) was stirred under reflux with diethylamine (150 c.c.). The original compound dissolved and a white crystalline solid was deposited. The heating was continued for 1 hour and the mixture was filtered. The solid (8·1 g.) was diethylamine hydrobromide, plates (from ethanol–ether), m. p. 212° unchanged on admixture with the authentic material. The diethylamine filtrate was evaporated nearly to dryness under reduced pressure and the residue treated with water. The insoluble material (halogen-free) was purified by dissolution in acid and reprecipitation with alkali as the pseudo-base (II). It was washed with water and dried in a vacuum over phosphoric oxide and then dissolved in warm ethanolic hydrogen chloride, and warm ether was added to crystallisation point. The *salt* was obtained as yellow needles, which were recrystallised from ethanol–ether [12·5 g., 64%; m. p. 192—194° (decomp.)] (Found : C, 63·0; H, 6·8; N, 8·9; Cl, 10·9. $C_{32}H_{40}O_4N_4Cl_2$ requires C, 62·4; H, 6·5; N, 9·1; Cl, 11·5%).

2 : 7-*Diamino*-10-3′-*diethylaminopropyl*-9-*phenylphenanthridinium Iodide Hydriodide*.—The foregoing salt (8 g.) was added to sulphuric acid (16 c.c.) and, when the liberation of hydrogen chloride ceased, water (8 c.c.) was added. The solution was slowly stirred at 130°, for 30 minutes, and then cooled. It was poured into ice and water, and the pH of the resulting solution raised to 7·3 by addition of ammonia solution. On addition of potassium iodide a red solid was deposited. Crystallisation from water containing a little potassium iodide gave the dihydrate of the *salt* as red needles [5·5 g., 60%; m. p. 180—201° (decomp.)] (Found : C, 45·5; H, 5·2; N, 8·2; I, 36·2; $H_2O$, 5·6. $C_{26}H_{32}N_4I_2,2H_2O$ requires C, 45·2; H, 5·2; N, 8·1; I, 36·7; $H_2O$, 5·2%).

2 : 7-*Biscarbethoxyamino*-10-3′-*diethylaminopropyl*-9-*phenylphenanthridinium Hydrogen Sulphate* 3′-*Methomethylsulphate*.—2 : 7-Biscarbethoxyamino-10-3′-diethylaminopropyl-9 : 10-dihydro-9-hydroxy-9-phenylphenanthridine [pseudo-base (II)] (6 g.) was dissolved in ethyl acetate (100 c.c.) and ethanol (60 c.c.), and methyl sulphate (2·8 c.c., 2·5 mols.) was added. The solution was refluxed for 30 minutes and cooled but no deposit formed. It was accordingly diluted with benzene–ether, to give a yellow solid *salt* which crystallised from ethanol–ethyl acetate as

yellow acicular prisms (6·3 g.), m. p. indefinite, *ca.* 250° (decomp.) (Found: C, 53·7; H, 6·1; N, 7·4. $C_{34}H_{46}O_{12}N_4S_2$ requires C, 53·3; H, 6·0; N, 7·3%).

2 : 7-*Diamino*-10-3′-*diethylaminopropyl*-9-*phenylphenanthridinium Iodide Methiodide*.—A solution of the foregoing compound in sulphuric acid (10 c.c.) and water (5 c.c.) was heated at 130° for 30 minutes and the product isolated as a *di-iodide* by the usual procedure. It crystallised from water containing potassium iodide, in red needles, m. p. 210—230° (decomp.), depressed on admixture with 2 : 7-diamino-10-3′-diethylaminopropyl-9-phenylphenanthridinium iodide hydriodide (3·6 g., 92%) (Found: C, 48·1; H, 5·0; N, 8·3; I, 37·2. $C_{27}H_{34}N_4I_2$ requires C, 48·5; H, 5·1; N, 8·4; I, 38·0%).

*Reaction between* 2 : 7-*Dinitro*-9-*phenylphenanthridine and Ethyl Toluene*-p-*sulphonate*.—2 : 7-Dinitro-9-phenylphenanthridine was heated with ethyl toluene-*p*-sulphonate, the quantity of ester, time of heating and temperature being varied. The mixture when cold was treated with dry ether, which was then decanted from the precipitated gum. The ethereal extract was shaken with a known volume of dilute alkali, and the alkaline layer back-titrated with standard acid to determine the amount of free toluene-*p*-sulphonic acid present. The value so obtained did not represent the exact amount formed as some acidity would probably be lost through pyrolysis of the acid. The residue from the ether-treatment was extracted with boiling water, and the extract, after cooling, was made alkaline (pH >8) to precipitate the red pseudo-base (III), which was collected and weighed. The residue from the aqueous extract from experiments with reaction times of 4 hours or less consisted of practically pure 2 : 7-dinitro-9-phenylphenanthridine (m. p., mixed m. p. with authentic material, and analysis), but, in those experiments where the decomposition had set in with a more prolonged heating, the residues were charred and the phenanthridine was then extracted with hot 2-ethoxyethanol.

The results obtained when the phenanthridine was heated with 5 mols. of ester at 185° have been tabulated. The figures in parentheses indicate the estimated amount of decomposition of the ester (mols.) when the phenanthridine was omitted in the above experiments.

| Time of heating (hours) | Mols. of ester decomposed (approx.) (= free acid titre) | Phenanthridine: Recovered (%) | Phenanthridine: Converted into pseudo-base (%) |
|---|---|---|---|
| 2 | 0 (0·15) | 65 | 30 |
| 3 | *ca.* 0 (0·45) | 51 | 43 |
| 4 | 0·12 (1·0) | 31 | 61 |
| 5 | 0·3 | 16 | 68 |
| 6 | 0·48 (1·7) | 5 | 71 |
| 7 | — | *ca.* 0 | 53 |
| 8 | 2·5 | 0 | 33 |
| 9 | — | 0 | *ca.* 10 |

2 : 7-*Diamino*-10-*ethyl*-9-*phenylphenanthridinium Bromide* (*Alternative Method of Preparation*).—2 : 7-Dinitro-9-phenylphenanthridine (20 g.) and ethyl toluene-*p*-sulphonate (60 g., *ca.* 5 mols.) were heated together under slow stirring for 4½ hours at 185°. The viscous solution was cooled and diluted with toluene or ether. The viscous residue was extracted with aqueous alcohol acidified with sulphuric acid. The residue from this extraction consisted of 2 : 7-dinitro-9-phenylphenanthridine (7·4 g., 37% recovery; m. p. 271—272°, unchanged on admixture with authentic material) (Found: N, 12·3. Calc. for $C_{19}H_{11}O_4N_3$: N, 12·2%). The hot aqueous-alcoholic extract was added to a hot stirred suspension of reduced iron (13 g.) in water (400 c.c.), and the mixture stirred under reflux for 4 hours. It was cooled, sodium hydroxide solution (40% w/w) added, and the pseudo-base ethyl ether extracted with toluene. The nearly black toluene layer was shaken with dilute hydrochloric acid solution, the pH of the acid layer was then raised to 7, and the required product salted out by addition of ammonium bromide. It crystallised from aqueous alcohol containing ammonium bromide as dark red plates (9·5 g.), m. p. 246—248°, raised to 248—250° by recrystallisation from methanol (Found, in material dried for 2 hours at 150°/1 mm.: N, 10·75; Br, 20·05. $C_{21}H_{20}N_3Br$ requires N, 10·65; Br, 20·3%).

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## 586. *A Confirmation of the Structure of 2 : 6-Dimethyl-4-pyrimidylmethyl-lithium and Some Observations on the Condensation of 1 : 3-Diketones with Acetamidine.*

By John C. Roberts.

4-Ethyl-2 : 6-dimethyl- and 2 : 4 : 5 : 6-tetramethyl-pyrimidine have been synthesised by unequivocal methods. Homologation of 2 : 4 : 6-trimethylpyrimidine (*via* the lithium compound) yielded a product whose derivatives were virtually identical with those from 4-ethyl-2 : 6-dimethylpyrimidine thus confirming the structure of 2 : 6-dimethyl-4-pyrimidylmethyl-lithium (Heyes and Roberts, *J.*, 1951, 328).

In a previous communication (Heyes and Roberts, *J.*, 1951, 328) it was shown that 2 : 4 : 6-trimethylpyrimidine, by reaction with phenyl-lithium, yielded a lithium derivative. This derivative reacted normally with *n*-dodecyl bromide to produce a pyrimidine having a long-chain alkyl substituent. Reduction and hydrolysis of the last-mentioned compound yielded acetaldehyde, proving that the original pyrimidine had not been attacked at the 2-position. The lithium derivative (A) was then formulated, on the basis of theoretical considerations, as 2 : 6-dimethyl-4-pyrimidylmethyl-lithium and the final product (B) as 2 : 6-dimethyl-4-*n*-tridecylpyrimidine. It was suggested by one of the referees of the above paper that an alternative structure for A, 2 : 4 : 6-trimethyl-5-pyrimidyl-lithium (and hence a structure 5-*n*-dodecyl-2 : 4 : 6-trimethylpyrimidine for B), could not be ignored. Although, from a theoretical standpoint (*loc. cit.*; see also Roberts, *Chem. and Ind.*, 1947, **66**, 658), we considered these alternative structures to be very unlikely it was thought desirable, before proceeding with further work in this field (forthcoming paper by Heyes and Roberts), to produce experimental confirmation of the original formulations. This paper describes the work undertaken for this purpose.

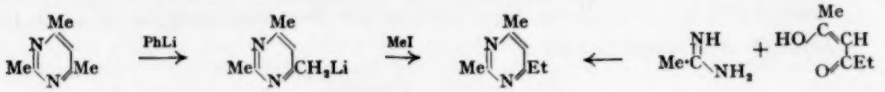
Although it was realised that many pyrimidines can readily be nitrated in an initially unsubstituted 5-position, no attempt was made to confirm the structure of B (and hence of its precursor, A) by this method since it is known (Gabriel and Colman, *Ber.*, 1902, **35**, 1570) that certain 4(6)-methylpyrimidines are attacked by nitric acid to yield furazan derivatives. Two methods for confirming the structure of A were explored but only one proved successful.

In the first there was envisaged a proof of the structure of A by identification of the oxidation product produced from its derivative B. This would have been pyrimidine-2 : 4 : 6-tricarboxylic acid or, if A and B had had the alternative structures mentioned above, pyrimidine-2 : 4 : 5 : 6-tetracarboxylic acid. Of these two acids the former had been previously characterised (Kondo and Yanai, *J. Pharm. Soc. Japan*, 1937, **57**, 173; *Chem. Zentr.*, 1938, II, 858) and it was thought that the structure of the latter could have been confirmed through its conversion into pyrimidine-5-carboxylic acid by partial decarboxylation (cf. Gabriel and Colman, *Ber.*, 1904, **37**, 3647). However, experiments on 2 : 4 : 6-trimethylpyrimidine indicated that the oxidation of such alkylpyrimidines (by means of potassium permanganate) was exceedingly slow. After a reaction period of 33 hours the only pure product isolable was a methylpyrimidinedicarboxylic acid, m. p. 196° (decomp.), which is probably 6-methylpyrimidine-2 : 4-dicarboxylic acid (cf. Ochiai and Yanai, *J. Pharm. Soc. Japan*, 1938, **58**, 76; *Chem. Zentr.*, 1938, II, 4242). This approach was abandoned.

The second method consisted in conversion of 2 : 4 : 6-trimethylpyrimidine (*via* its lithium derivative) into its next higher homologue and comparison of the latter with the known 4-ethyl-2 : 6-dimethyl- and 2 : 4 : 5 : 6-tetramethyl-pyrimidine. The material produced by homologation could not be obtained analytically pure * but it yielded derivatives (a picrate, a picrolonate, and a double compound with mercuric chloride) which were

* This material was probably contaminated with, *inter alia*, some 4 : 6-diethyl-2-methylpyrimidine; cf. the production of some 2-methyl-4 : 6-di-*n*-tridecylpyrimidine as a by-product in the preparation of 2 : 6-dimethyl-4-*n*-tridecylpyrimidine (Heyes and Roberts, *J.*, 1951, 330).

virtually identical with the corresponding derivatives prepared from 4-ethyl-2 : 6-dimethylpyrimidine. The following reactions were thus established :




The structures of 2 : 6-dimethyl-4-pyrimidylmethyl-lithium and 2 : 6-dimethyl-4-*n*-tridecylpyrimidine are, therefore, confirmed.

*Condensation of* 1 : 3-*Diketones with Acetamidine.*—Previous attempts to condense a 1 : 3-diketone with an amidine appear to have been limited almost entirely to the use of acetylacetone. [Pinner found that formylacetone would not condense with benzamidine (*Ber.*, 1893, **26**, 2122). Valerylacetone and urea are stated to produce diuramidovalerylacetone and not the expected pyrimidine (Yanai and Naito, *J. Pharm. Soc. Japan*, 1941, **61**, 46; *Chem. Zentr.*, 1942, I, 483).] It is now shown that propionylacetone will condense with acetamidine under conditions similar to those described by Bowman for acetylacetone (*J.*, 1937, 494; *cf.* Kondo and Yanai, *loc. cit.*)—conditions which are probably optimum for condensations of this type (Haley and Maitland, *J.*, 1951, 3160). The condensation gave a moderate yield of the desired 4-ethyl-2 : 6-dimethylpyrimidine. Methylacetylacetone (3-methylpentane-2 : 4-dione) and acetamidine yielded a small quantity of 2 : 4 : 5 : 6-tetramethylpyrimidine which was purified and identified as the picrate.

The condensation of acetylacetone and acetamidine, by Bowman's method, was found to yield not only 2 : 4 : 6-trimethylpyrimidine but also a considerable quantity of acetylacetone imine. By a similar process propionylacetone yielded the corresponding pyrimidine and, as a by-product, an appreciable quantity of a monoimine of propionylacetone whose exact structure was not determined. In the condensation of methylacetylacetone with acetamidine there was no evidence for the production of a corresponding imino-compound.

## Experimental

2 : 4 : 6-*Trimethylpyrimidine.*—Acetylacetone (77 g.) was condensed with acetamidine by Bowman's method. The crystalline hydrate was filtered off. The filtrate was saturated with anhydrous potassium carbonate (300 g. required) and was then extracted with ether (3 × 100 c.c.). To the mixture of the ethereal extract and the crystalline hydrate was added, with occasional vigorous shaking, just sufficient anhydrous potassium carbonate to give two clear liquid layers. The ethereal solution was separated, dried ($K_2CO_3$, 25 g.) and filtered. The solvent was evaporated and the residue distilled at 12 mm. Three fractions were collected : (i) b. p. <62° (1 g.); (ii) b. p. 62—90° (18·6 g.); (iii) b. p. 94—120°, (9·5 g.). Fraction (ii) was redistilled and yielded, as a middle fraction, 12·2 g. of 2 : 4 : 6-trimethylpyrimidine, b. p. 68—72°/11·5 mm. (Kondo and Yanai, *loc. cit.*, give b. p. 80—84°/16 mm.), 168—170°/745 mm. [Bowman, *loc. cit.*, gives " b. p. 160° (approx.) "].

Fraction (iii) from the first distillation partly solidified at room temperature. It was kept at −2° overnight and the crystalline portion was then separated by filtration from some contaminating dark oil. The crystals were well washed with light petroleum (b. p. 40—60°) and dried (3·1 g.; m. p. *ca.* 34°) at room temperature in a vacuum over concentrated sulphuric acid. Distillation *in vacuo* of this crystalline material yielded a fraction, b. p. 98—99°/11·5 mm. (1·4 g.), which solidified in the condenser to a mass of colourless prisms. This substance, m. p. 39—40°, was readily soluble in water, ethanol, and benzene; it gave a dark-red ferric reaction in ethanol and was identified as acetylacetone imine (2-aminopent-2-en-4-one) (Found : C, 60·7; H, 9·0; N, 13·9. Calc. for $C_5H_9ON$ : C, 60·6; H, 9·2; N, 14·1%) (Combes and Combes, *Bull. Soc. chim.*, 1892, **7**, 779, give m. p. 43°).

*Oxidation of* 2 : 4 : 6-*Trimethylpyrimidine with Potassium Permanganate.*—A solution of the pyrimidine (1·0 g.) in water (50 c.c.) was gently boiled under reflux with vigorous mechanical stirring. An aqueous solution of potassium permanganate (2% w/v) was added at a rate such that there was always a slight excess of the oxidising agent in the reaction mixture. During 33 hours 350 c.c. of the permanganate solution were added. Reaction then appeared to be complete. The manganese dioxide was filtered off and washed thoroughly. The pH of the combined filtrate and washings was adjusted to 8·5—9·0 by the addition of dilute sulphuric acid, and the solution was then evaporated to 150 c.c. After filtration to remove a little manganese

dioxide and some gelatinous matter, the solution was strongly acidified (pH <1) with dilute sulphuric acid and set aside overnight. The crystalline precipitate was filtered off, washed with water, and dried [0·48 g.; m. p. 197° (decomp.)]. Recrystallisation from boiling water yielded 6(or 2)-methylpyrimidine-2(or 6) : 4-dicarboxylic acid as colourless, microscopic prisms which were dried (0·35 g.) *in vacuo* over concentrated sulphuric acid. The m. p. (with decomp.) of the dihydrate, thus prepared, varied with the rate of heating from 196° (slow heating) to 208° (rapid heating) (Found : C, 38·7; H, 4·8; N, 12·9%; equiv., by titration, 107·6. Calc. for $C_7H_6O_4N_2,2H_2O$ : C, 38·5; H, 4·6; N, 12·8%; equiv., 109·1). An aqueous solution of this acid was coloured wine-red by the addition of a solution of ferrous sulphate. Ochiai and Yanai (*loc. cit.*) record the m. p. of " 6-methylpyrimidine-2 : 4-dicarboxylic acid " (in the form of the dihydrate) as 195—197° (decomp.).

By evaporation of the neutralised mother-liquor of the foregoing acid, followed by acidification, there was obtained a further quantity of crystalline acidic material (*ca.* 0·18 g.) which, when heated, gradually decomposed over the range 190—360° and probably consisted of a mixture of the dicarboxylic acid with pyrimidine-2 : 4 : 6-tricarboxylic acid. This material was not further investigated.

4-*Ethyl*-2 : 6-*dimethylpyrimidine*.—A mixture of a solution of anhydrous potassium carbonate (27·6 g., 2 mols.) in water (90 c.c.) and propionylacetone (11·4 g., 1 mol.) [prepared by the method of Morgan and Reeves (*J.*, 1923, **123**, 447)] was not homogeneous after having been vigorously shaken. Acetamidine hydrochloride (9·45 g., 1 mol.) was added and the mixture was vigorously shaken, in a tightly-stoppered vessel, for 20 hours. After being kept at room temperature, with intermittent shaking, for 26 days the mixture was saturated with anhydrous potassium carbonate (*ca.* 60 g. required) and extracted with ether (45 + 45 + 30 c.c.). The combined ethereal extracts were washed with water (15 + 10 c.c.) and then extracted thoroughly with 2N-sodium hydroxide (40 + 40 + 30 c.c.) in order to remove any unchanged diketone. The ethereal layer was washed with water (10 c.c.), dried ($Na_2SO_4$, 20 g.), filtered, and evaporated to about 15 c.c. This concentrated solution, after being kept over pellets of potassium hydroxide (1 g.) for 20 minutes, was filtered through an alkali-resistant filter-paper and the ether was evaporated. The residue, when distilled *in vacuo*, yielded fractions (i) b. p. 72—76°/11 mm. (1·6 g.), and (ii) b. p. 102—104°/11 mm. (*ca.* 0·5 g.).

The first fraction, 4-*ethyl*-2 : 6-*dimethylpyrimidine* (Found, on a redistilled specimen : C, 70·9; H, 8·9; N, 20·3. $C_8H_{12}N_2$ requires C, 70·6; H, 8·9; N, 20·6%), was a colourless, mobile, volatile, hygroscopic oil with an intense odour somewhat resembling that of impure acetamide. It was soluble in water and in the common organic solvents.

This pyrimidine (0·3 g.) was gently warmed with picric acid (0·5 g.) in 95% ethanol (3·5 c.c.), until a clear solution was obtained. After being cooled and " scratched " the solution deposited 0·50 g. of crude material, m. p. 85—87°. Recrystallisation from 95% ethanol (1·7 c.c.) yielded the pure *picrate* (0·04 g.), lemon-yellow prisms, m. p. 86—87° (Found : N, 19·3. $C_8H_{12}N_2,C_6H_3O_7N_3$ requires N, 19·2%), very readily soluble in water, ethanol, and benzene. The *picrolonate* separated from ethanolic solution as a yellow micro-crystalline powder, m. p. 191° (decomp.) (Found : N, 21·2. $C_8H_{12}N_2,C_{10}H_8O_5N_4$ requires N, 21·0%). An aqueous solution of the pyrimidine, after being mixed with a solution of mercuric chloride, slowly deposited silky needles of the double *compound*, m. p. 103—104° (Found : N, 4·2. $C_8H_{12}N_2,2HgCl_2$ requires N, 4·1%).

The second fraction from the distillation crystallised in the condenser. The crystals were collected, washed well with light petroleum (b. p. 40—60°), and dried (*ca.*, 80 mg.; m. p. 58—60°). Recrystallisation from light petroleum (b. p. 40—60°) yielded 2-aminohex-2-en-4-one (or 3-aminohex-3-en-5-one) as colourless prisms, m. p. 62° (Found : C, 64·1; H, 9·3; N, 12·1. Calc. for $C_6H_{11}ON$ : C, 63·7; H, 9·8; N, 12·4%). This material was sparingly soluble in water and light petroleum and gave a dark-red ferric reaction in ethanol. (Küster, *Chem. Zentr.*, 1925, I, 2080, records m. p. 49° for 2-aminohex-2-en-4-one.)

2 : 4 : 5 : 6-*Tetramethylpyrimidine Picrate*.—A mixture of a solution of anhydrous potassium carbonate (29·8 g., 2 mols.) in water (100 c.c.) and methylacetylacetone (12·3 g., 1 mol.) (Perkin, *J.*, 1892, **61**, 848) was not homogeneous after having been vigorously shaken. Acetamidine hydrochloride (10·2 g., 1 mol.) was added and the mixture was vigorously shaken for 24 hours. After being kept at room temperature, with occasional shaking, for 7 weeks the mixture was saturated with potassium carbonate (*ca.* 70 g.), and the crude product was isolated by the method described for 4-ethyl-2 : 6-dimethylpyrimidine. The residue (1—2 c.c.), produced by evaporation of the dried ethereal extract, was distilled *in vacuo*. A brown oil (0·5 g.) slowly distilled at 82—122°/20 mm. (The distillation was too slow for an accurate estimation of b. p.

to be made.) The odour of this oil was very similar to that of 4-ethyl-2 : 6-dimethylpyrimidine. To a warm (60°) solution of this oil (0·5 g.) in 95% ethanol (3 c.c.) was added a warm solution of picric acid (0·75 g.) in 95% ethanol (6 c.c.). The solution, having been cooled, " scratched " and allowed to stand overnight at −2°, deposited greenish-yellow crystals which were filtered off, washed and dried (0·7 g., m. p. 117—128°). This material, after three recrystallisations from ethanol (95%), yielded 2 : 4 : 5 : 6-*tetramethylpyrimidine picrate* (0·48 g.) as deep-yellow prisms, m. p. 129—130° [Found : C, 46·6; H, 4·1; N, 19·3; picric acid (by precipitation as nitron picrate, Busch and Blume, *Z. angew. Chem.*, 1908, **21**, 354), 62·6%. $C_8H_{12}N_2,C_6H_3O_7N_3$ requires C, 46·0; H, 4·1; N, 19·2; picric acid, 62·7%].

*Homologation of* 2 : 4 : 6-*Trimethylpyrimidine.*—A phenyl-lithium solution was prepared from bromobenzene (15·7 g., 1 mol.), lithium chips (1·42 g., 2·04 atomic equivs.), and dry ether (70 c.c.) under nitrogen. To the ice-cold solution was slowly added, with mechanical stirring, 2 : 4 : 6-trimethylpyrimidine (12·2 g., 1 mol.) in dry ether (25 c.c.). The mixture was stirred for 15 minutes at room temperature and was then cooled in a freezing-mixture. Methyl iodide (15·6 g., 1·1 mols.) in dry ether (10 c.c.) was slowly added with vigorous stirring. The greenish-yellow sludge of the lithium derivative gradually disappeared. The mixture was boiled under reflux for 2 hours. After being kept overnight at room temperature the mixture was vigorously stirred with water (40 c.c.). The lower layer, after being extracted with ether (20 c.c.), was discarded. The combined ethereal solutions were extracted with 2N-hydrochloric acid (60 + 40 + 20 c.c.). To the combined acid extracts was added, with cooling, a solution of sodium hydroxide (12 g.) in water (40 c.c.), whereupon a yellow oil separated. The mixture was extracted with ether (100 + 50 c.c.), and the ethereal extract washed with water (20 c.c.), dried ($MgSO_4$, 8 g.), and filtered. After evaporation of the ether the residue was distilled at 13 mm. and yielded fractions (i) b. p. 73—77° (0·55 g.), (ii) b. p. 77—81° (3·1 g.), and (iii) b. p. 82—86° (0·85 g.). The middle fraction was used for preparing the following derivatives. 0·3 G. of the base, when treated as described for 4-ethyl-2 : 6-dimethylpyrimidine, yielded 0·45 g. of crude picrate, m. p. 85—87°. Recrystallisation from 95% ethanol gave 0·33 g. of lemon-yellow prisms (Found : N, 19·1%), m. p. 87—88° alone, or, after admixture with an authentic specimen of 4-ethyl-2 : 6-dimethylpyrimidine picrate, 87°.

The picrolonate separated from ethanolic solution as a microcrystalline, yellow powder, m. p. 192° decomp.).

The double compound with mercuric chloride separated from water in silky needles, m. p. 105° alone, or, after admixture with an authentic specimen of the double compound from 4-ethyl-2 : 6-dimethylpyrimidine, 104—105°. (The m. p.s of these double compounds with mercuric chloride vary with their " age." The constants quoted above were for specimens of " age " one day.)

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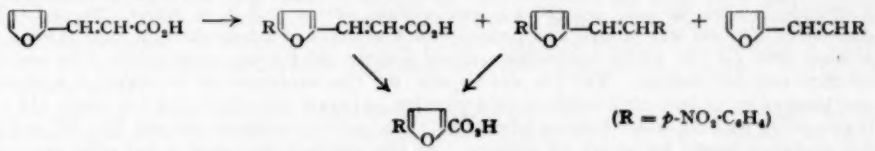
## 587. *The Reaction of Diazonium Salts with Furylacrylic Acid. Part I.*

By WALTER FREUND.

The reaction of diazonium salts with furylacrylic acid leads mainly to diarylated compounds (5-aryl-2-styrylfurans). As by-products, 2-styrylfurans and β-(5-aryl-2-furyl)acrylic acids are obtained (Austr. P. 26,725/1949).

As 4-aminostilbene derivatives have been shown to be carcinogenic as well as to have an inhibitory effect on the development of transplanted tumours in the rat, the preparation of compounds in which one benzene nucleus is replaced by a heterocyclic ring such as furan or thiophen was considered of interest. These experiments were carried out in 1946—1947. In the meantime, Brown and Kon (*J.*, 1948, 2150), with the same object, prepared a considerable number of such compounds, amongst them 2-4′-aminostyrylfuran, which they synthesised according to the Meerwein method. *E.g.*, they treated diazotised *p*-nitroaniline and furylacrylic acid in acetone with a sodium acetate buffer in presence of cupric chloride; they obtained only 2-4′-nitrostyrylfuran, in agreement with other

reactions of diazonium compounds on αβ-unsaturated carbonyl compounds (Meerwein, Buchner, and van Emster, *J. pr. Chem.*, 1939, **152**, 237; B.P. 480,617). However, this furan is only one of the reaction products: the main product is a diarylated compound, (5?)-4′-nitrophenyl-2-4′-nitrostyrylfuran (Austr. P. 26,725/1949), and an acid of m. p. 262° is also obtained. Oxidation of the diarylated compound with potassium permanganate gave *p*-nitrobenzoic acid and, probably, 5-*p*-nitrophenyl-2-furoic acid. The last acid was obtained by oxidation of the acid of m. p. 262° which is consequently considered to be β-(5-*p*-nitrophenyl-2-furyl)acrylic acid. The relations of the various compounds are shown in the annexed scheme.

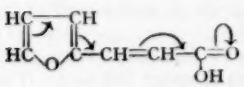



On the other hand, diazotised *o*-nitroaniline and furylacrylic acid have so far yielded only one product, an acid. Attempts at decarboxylation gave only resinous masses. If this acid were *trans*-β-2-furyl-α-*o*-nitrophenylacrylic acid, in accordance with the results of Meerwein *et al.* (*loc. cit.*), it should be decarboxylated to *trans*-1-2′-furyl-2-*o*-nitrophenylethylene in the same way as is the isomeric *trans*-α-2′-furyl-*o*-nitrocinnamic acid (Amstutz and Spitzmiller, *J. Amer. Chem. Soc.*, 1943, **65**, 367). The acid has therefore been assigned the structure β-2-furyl-α-*o*-nitrophenylacrylic acid. Oxidation experiments were inconclusive, as no arylfuroic acid could be isolated.

*p*-Chloroaniline and ethyl *p*-aminobenzoate gave results in agreement with those obtained with *p*-nitroaniline, although the product from the latter could not be crystallised. The diarylated compounds, 5-*p*-chlorophenyl-2-4′-chlorostyrylfuran and 5-*p*-carbethoxyphenyl-2-4′-carbethoxystyrylfuran, gave a deep blue fluorescence in benzene as did the diamine obtained from the dinitro-compound. This amine has, however, not yet been closely investigated.

Sulphanilic and arsanilic acid each yielded only one product, probably the 5-substituted furylacrylic acid (B.P. appln. 7144/1948). The corresponding diarylated compounds could not be purified although they were undoubtedly formed.

Introduction of two aryl groups by diazo-compounds has so far only been observed with quinones (D.R.-P. 508,395; Kvalnes, *J. Amer. Chem. Soc.*, 1934, **56**, 2478). That the 5-position in furylacrylic acid is the reactive one has already been observed by Gilman and Wright (*ibid.*, 1930, **52**, 3349) who found that ethyl furylacrylate yields a dibromo-compound, which loses hydrogen bromide to yield ethyl β-(5-bromo-2-furyl)acrylate. Marckwald (*Ber.*, 1887, **20**, 2813) found that furylacrylic acid when treated in alcohol with hydrogen chloride yields diethyl γ-ketopimelate; the ease with which the furan nucleus is opened and water added, is not met in other furan derivatives. Furylacrylic acid does not undergo the Diels–Alder reaction in spite of its three conjugated double bonds (van Campen and Johnson, *J. Amer. Chem. Soc.*, 1933, **55**, 430), while furylethylene according to Paul (*Compt. rend.*, 1939, **208**, 1028) adds maleic anhydride between the β-atom of the furan nucleus and the ethylene group. More recently, Herz (*J. Amer. Chem. Soc.*, 1946, **68**, 2732) showed that if the conjugation of furylacrylic acid is destroyed by hydrogenation, as in furylpropionic acid, then a normal Diels–Alder reaction takes place on the furan nucleus. According to Herz, the electronic displacements are represented as inset. A true diene, such as butadiene, adds only one aryl group (Goyner and Ropp, *ibid.*, 1948, **70**, 2283). There can therefore be little doubt that the peculiar polarisation of the double bonds in furylacrylic acid is responsible for the double arylation of this compound which has not been met before in the numerous applications of the Meerwein reaction. It is not in agreement with the theory that organic radicals are involved in the Meerwein reaction as claimed by Waters (*J.*, 1946, 411; "The Chemistry of Free Radicals," London, 1946). The results of reaction



between diazotised amines and furylacrylic acid support Meerwein's suggestion that the polarisation of the double bonds is the governing factor.

Further results with furylacrylic acid, and results with furyl-pentadienoic and -heptatrienoic acid, as well as with β-thienylacrylic acid, will be reported later.

## Experimental

M. p.s are uncorrected.

*Furylacrylic Acid and* p-*Nitroaniline.*—*p*-Nitroaniline (13·8 g.) was diazotised with 25% hydrochloric acid (43 c.c.), ice (20 g.), and sodium nitrite (7·3 g. in water, 20 c.c.). The diazonium solution was filtered and poured into a solution of β-2-furylacrylic acid (13·8 g.) in acetone (200 c.c.) in which crystalline sodium acetate (40 g.) was suspended. The reaction mixture was well shaken. The pH was approx. 6. Gas evolution set in almost immediately and became so violent after addition of a solution of cupric chloride (4·2 g.) in water (12 c.c.) that cooling with ice-water became advisable. The reaction temperature was 23—24° and the gas evolution lasted for about 20 minutes. As the reaction proceeded a red solid separated which was filtered off (6·2 g.) and washed with dilute aqueous ammonia, water, and alcohol. This material melted at 220—225°. It was heated with a small quantity of acetone and allowed to cool. The solid material was filtered off and, recrystallised from acetic acid, formed small red needles, m. p. 234°, which dissolved in benzene with strong green fluorescence. After about 3 weeks in daylight the green fluorescence disappeared. This compound is 5-p-*nitrophenyl*-2-4′-*nitrostyrylfuran* (Found: C, 64·5, 64·5; H, 3·6, 3·7; N, 8·1, 8·3. $C_{18}H_{12}O_5N_2$ requires C, 64·4; H, 3·6; N, 8·3%). It dissolved in concentrated sulphuric acid with a wine-red colour which changed rapidly to dark purple.

The aqueous-acetone mother-liquor was steam-distilled. This removed *p*-chloronitrobenzene. The dark resinous residue was separated from the hot liquor which contained the bulk of unchanged furylacrylic acid, digested with dilute cold sodium hydrogen carbonate solution, and extracted 3 times with dilute aqueous ammonia. From the united filtered ammoniacal solutions, hydrochloric acid precipitated orange-coloured β-(5-p-*nitrophenyl*-2-*furyl*)*acrylic acid* which was filtered off and recrystallised from acetic acid from which it separated in thick brown prisms, m. p. 262° (3 g.), which dissolved in concentrated sulphuric acid with a golden yellow colour (Found: C, 60·3, 60·3; H, 3·6, 3·5; N, 5·4. $C_{13}H_9O_5N$ requires C, 60·2; H, 3·5; N, 5·3%). It did not fluoresce in any solvent.

The material remaining after the ammoniacal extraction was dried and extracted 3 times with absolute alcohol (150 c.c.), which was allowed to evaporate at room temperature. A brown mass, still somewhat resinous, remained. After two recrystallisations from light petroleum (b. p. 60—80°) and absolute alcohol, brown needles, m. p. 130—131°, of 2-*p*-nitrostyrylfuran were obtained (Brown and Kon, *loc. cit.*, give m. p. 130—131°) (Found: C, 67·1, 67·2; H, 4·3, 4·3; N, 6·6. Calc. for $C_{12}H_9O_3N$: C, 67·0; H, 4·2; N, 6·5%).

*Oxidation of* 5-p-*Nitrophenyl*-2-4′-*nitrostyrylfuran.*—5-*p*-Nitrophenyl-2-4′-nitrostyrylfuran (3·36 g.) in acetone (200 c.c.) containing crystalline magnesium sulphate (7·5 g.) was oxidised with potassium permanganate (4·5 g.), and then, as oxidation was incomplete, with acetone (100 c.c.), magnesium sulphate (2·5 g.), and potassium permanganate (1·5 g.). The furan nucleus was partly oxidised. The acetone was allowed to evaporate and the dark material freed from manganese dioxide with sulphur dioxide. A light yellow material (2·65 g.) was filtered off and extracted with cold dilute sodium hydrogen carbonate solution which removed *p*-nitrobenzoic acid (2·25 g.; m. p. and mixed m. p. 238—239°).

The reddish-brown solid insoluble in cold bicarbonate solution (0·4 g.) was twice recrystallised from absolute alcohol, yielding β-(5-p-*nitrophenyl*)-2-*furoic acid*, m. p. 204—205° (Found: C, 56·8, 56·8; H, 3·1, 3·1; N, 6·0. $C_{11}H_7O_5N$ requires C, 56·7; H, 3·0; N, 6·0%).

*Oxidation of* β-(5-p-*Nitrophenyl*-2-*furyl*)*acrylic Acid.*—β-5-*p*-Nitrophenylfurylacrylic acid (2·62 g.), when oxidised as above, gave 5-*p*-nitrophenyl-2-furoic acid, m. p. and mixed m. p. 204—205°.

*Furylacrylic Acid and* o-*Nitroaniline.*—*o*-Nitroaniline (6·9 g.) was diazotised with 25% hydrochloric acid (20—22 c.c.), ice (20 g.), and sodium nitrite (3·65 g.) in water (10 c.c.). The filtered solution was poured into a solution of furylacrylic acid (6·9 g.) in acetone (80 c.c.) containing crystalline sodium acetate (15—20 g.). The colour of the solution changed from orange-red to brown. There was brisk gas evolution. In spite of cooling, the temperature rose to 20°. Addition of a solution of cupric chloride (2 g.) in water (5 c.c.) did not revive

noticeably the gas evolution which lasted for only about 10—15 minutes. The acetone was allowed to evaporate overnight. The dark resinous residue was digested with dilute hydrochloric acid, water, and then dilute cold sodium hydrogen carbonate solution from which on acidification some unchanged furylacrylic acid was recovered. The dark material was then extracted with dilute ammonia solution which was filtered and acidified. The precipitate was washed with cold sodium hydrogen carbonate solution and on crystallistion from benzene yielded β-(5-o-*nitrophenyl-2-furyl)acrylic acid* as brown needles, m. p. 178° (2·7 g.) (Found: N, 5·3, 5·4. $C_{13}H_9O_5N$ requires N, 5·3%).

*Furylacrylic Acid and* p-*Chloroaniline.*—*p*-Chloroaniline (12·75 g.), diazotised as above, was treated with furylacrylic acid (13·8 g.) at pH 6. Gas evolution set in at once and became violent after addition of a solution of cupric chloride (3 g.) in water (10 c.c.), but ceased after approx. 40 minutes. Working up, as above, yielded from the ammoniacal solution, a small amount of β-(5-p-*chlorophenyl-2-furyl)acrylic acid*, m. p. 196—198° (Found: Cl, 14·5. $C_{13}H_9O_3Cl$ requires Cl, 14·3%).

The dark ammonia-insoluble material was steam-distilled. 2-p-*Chlorostyrylfuran* which volatilised crystallised from light petroleum as yellowish rods, m. p. 97—98° (Found: C, 70·5, 70·6; H, 4·4, 4·5; Cl, 17·3. $C_{12}H_9OCl$ requires C, 70·4; H, 4·4; Cl, 17·4%).

The material involatile in steam was extracted with 3 × 200 c.c. of hot light petroleum (b. p. 70—90°). From these extracts small amounts of crystalline material were obtained of varying m. p. (118° to 125°). The mother-liquor after evaporation deposited material, from which crystallisation from methyl alcohol yielded a little 5-p-*chlorophenyl-2-4′-chlorostyryl-furan*, m. p. 135—136° (Found: C, 68·7, 68·7; H, 3·9, 3·9; Cl, 22·4. $C_{18}H_{12}OCl_2$ requires C, 68·6; H, 3·8; Cl, 22·5%).

A similar experiment (with Dr. A. Komzak), benzene being used instead of light petroleum for extraction, gave, as purest compound, material of m. p. 139—140°.

*Furylacrylic Acid and Ethyl* p-*Aminobenzoate* [with E. R. Stove].—Ethyl *p*-aminobenzoate (8·25 g.), diazotised in the usual way, was added to a solution of furylacrylic acid (6·9 g.), etc., as above. Gas evolution set in at once, was fastest after approx. 7 minutes, and then soon ceased. No cupric chloride was used. The mixture was poured into aqueous sodium hydrogen bicarbonate (15 g. in 150 c.c.), and the acetone was allowed to evaporate. The solution was filtered and the solid washed with water and cold dilute aqueous ammonia, and then extracted with dilute aqueous ammonia at 50°. Acidification yielded (?)β-(5-p-*carbethoxy-2-furyl)acrylic acid* as a brown solid which crystallised from benzene in sheafs of buff-coloured needles, m. p. 202—203° (2 g.) (Found: C, 67·4, 67·1; H, 5·1, 5·0; EtO, 15·6. $C_{16}H_{14}O_5$ requires C, 67·1; H, 4·9; EtO, 15·7%).

The residue from the ammonia extraction was dried in a vacuum-desiccator and then extracted with methyl alcohol (500 c.c.), which on evaporation left crystals embedded in a resin. The crystals were separated mechanically, washed with a small amount of acetone, and recrystallised from absolute alcohol, yielding prisms, m. p. 135—140°. After a further recrystallisation from methyl alcohol, prisms with a golden sheen were obtained having m. p. 144° and soluble in benzene with a gentian-blue fluorescence. This compound is 2-p-*carboethoxyphenyl-5-4′-carbethoxystyrylfuran* (Found: EtO, 23·2. $C_{24}H_{22}O_5$ requires EtO, 23·1%).

*Furylacrylic Acid and Sulphanilic Acid.*—Diazotised sulphanilic acid (17·3 g.) was treated with furylacrylic acid (13·8 g.) at pH approx. 6. Cupric chloride was added. Gas evolution became rapid at 18—20° and lasted for about 45 minutes. After a few hours, the mixture was steam-distilled and then allowed to cool. A solid crystallised which was filtered off and washed with cold alcohol which removed unchanged furylacrylic acid. β-(5-p-*Sulphophenyl-2-furyl)-acrylic acid* (1·2 g.) remained as a grey solid which crystallised from water in rods and plates, m. p. >300° (Found: C, 53·2, 53·1; H, 3·5, 3·3. $C_{13}H_{10}O_6S$ requires C, 53·1; H, 3·4%). Concentration of the mother-liquor gave crystals which fluoresced blue in acetic (characteristic of the diarylated compounds), but no pure material free from nitrogen was obtained.

I thank Dr. K. F. Tettweiler, formerly of the University of Melbourne, for the microanalyses. This work was supported in part by a grant from the National Council of Health and Medical Research, Canberra.

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## 588. *A New Synthesis of Arsonic Acids. Part II.* Coupling of αβ-Unsaturated Carbonyl Compounds with Diazotised Arsanilic Acid and 4-Amino-2-nitrophenylarsonic Acid.*

By Walter Freund.

4-Hydroxy-3-nitrocinnamic acid and diazotised arsanilic acid give 4′-hydroxy-3′-nitrostilbene-4-arsonic acid. 4-Hydroxycinnamic acid and diazotised 4-amino-2-nitrophenylarsonic acid give 4′-hydroxy-3-nitrostilbene-4-arsonic acid.

The arsonic acids described in Part I * contain neither amino- nor nitro-groups. For therapeutic purposes it seemed desirable to prepare hydroxynitro-arsonic acids which could then be reduced to the corresponding amino-compounds. Meerwein, Buchner, and van Emster (*J. pr. Chem.*, 1939, **152**, 237) failed in attempts to couple *p*-nitrocinnamic acid with diazo-compounds. l'Ecuyer, Turgotte, Giguère, Olivier, and Roberge succeeded in coupling *m*- (*Canad. J. Res.*, 1947, **25**, 575) and *p*-nitrocinnamic acid (*ibid.*, 1948, **26**, 70) with various diazo-compounds by using large amounts of acetone to keep the nitrocinnamic acids in solution. In order to obtain the 3-amino-4-hydroxy-configuration present in many of the organic arsenicals, 4-hydroxy-3-nitrocinnamic acid was prepared. Its melting point is given by Einhorn and Grabfield (*Annalen*, 1888, **243**, 374) as 198° but by Johnson and Kohmann (*J. Amer. Chem. Soc.*, 1915, **37**, 166) as 223°. The acid has now been synthesised by a Doebner condensation on 4-hydroxy-3-nitrobenzaldehyde, which was prepared by nitration of *p*-hydroxybenzaldehyde (Paal, *Ber.*, 1895, **28**, 2415). After recrystallisation from alcohol, an acid of m. p. 221—223° was obtained.

l'Ecuyer *et al.* (*loc. cit.*) obtained 10% of stilbenes by coupling *m*- and *p*-nitrocinnamic acids with various diazotised amines. Unfortunately, diazotised arsanilic acid does not couple as well as the amines used by the Canadian workers. The yield of 4′-hydroxy-3′-nitrostilbene-4-arsonic acid is only about 2%; the main product is azobenzene-4 : 4′-diarsonic acid (Karrer, *Ber.*, 1912, **45**, 2362; Fichter and Elkind, *Ber.*, 1916, **49**, 246).

Coupling with 4-amino-2- or -3-nitrophenylarsonic acid was then considered. The latter, when diazotised and buffered with sodium acetate, exchanges the nitro- for a hydroxy-group (Benda, *Ber.*, 1911, **44**, 3579; D.R.-P. 243,648) and is therefore useless. The 2-nitro-acid is described only in D.R.-P. 267,307 (*Chem. Zentr.*, 1913, II, 2067). Its diazo-solution with *p*-hydroxycinnamic acid gave the expected 4′-hydroxy-3-nitrostilbene-4-arsonic acid but the yield was poor.

### Experimental

M. p.s are uncorrected.

*4-Hydroxy-3-nitrobenzaldehyde* (cf. Paal, *Ber.*, 1895, **28**, 2415).—*p*-Hydroxybenzaldehyde, m. p. 114—116° (24·4 g.), nitric acid (15 g.; *d* 1·48), and acetic acid (100 g.) were gently warmed until reaction set in, and cooled with ice-water when the reaction became violent. On cooling, 4-hydroxy-3-nitrobenzaldehyde crystallised (12 g., 37%). Recrystallisation from alcohol gave material of m. p. 143—144°.

*4-Hydroxy-3-nitrocinnamic Acid.*—4-Hydroxy-3-nitrobenzaldehyde (m. p. 140—142°; 20·8 g.) and malonic acid (31 g.) in dry pyridine (100—150 c.c.) were heated on the boiling water-bath until dissolved, and a few drops of piperidine were added. Evolution of carbon dioxide was rapid and after 2 hours the mixture was boiled over a naked flame for 20 minutes. After cooling, it was poured into an ice-cold 17% hydrochloric acid (400 c.c.). The 4-hydroxy-3-nitrocinnamic acid precipitated was purified by dissolution in sodium hydrogen carbonate solution, recovered, dried, and digested with acetone (150 c.c.). The insoluble material, recrystallised from alcohol, melted at 221—223° (Johnson and Kohmann give m. p. 223°) (yield : 18·8 g., 65·7%) (Found : N, 6·8. Calc. for $C_9H_7O_5N$ : N, 6·7%).

*4′-Hydroxy-3′-nitrostilbene-4-arsonic Acid.*—To arsanilic acid (10·85 g.) in a solution of sodium hydrogen carbonate (4·2 g.) in water (90 c.c.) was added sodium nitrite (3·65 g.) in water (10 c.c.), and the mixture was dropped into cooled 25% hydrochloric acid (32 c.c.). The clear diazonium solution was added to a solution made from 4-hydroxy-3-nitrocinnamic acid

* Part I, *J.*, 1951, 1943.

(10·45 g.) in acetone (550 c.c.) and sodium chloroacetate (35 g.) in water (25 c.c.). After the pH had become adjusted to about 5, the solution was yellow, and the temperature approx. 16°. The colour changed to red, the solution was warmed on the water-bath to 20—22°, and a solution of cupric chloride (10 g.) in water (10 c.c.) was added dropwise. Gas evolution set in and a reddish precipitate began to be formed. The gas evolution lasted for about 20 minutes. After a few hours, the precipitate was filtered off, washed with acetone, air-dried, and then dissolved in 2N-sodium hydroxide (100 c.c.). The alkaline solution was filtered from copper hydroxide and acidified with hydrochloric acid. The brown precipitate was filtered off, dried, and digested with cold dilute sodium hydrogen carbonate solution in which the greater part was soluble. The *4'-hydroxy-3'-nitrostilbene-4-arsonic acid* remaining undissolved was recrystallised from acetic acid and alcohol, from which it crystallised in clusters of yellow needles (*ca.* 2%) which did not melt at 300° (Found: C, 46·3, 45·9; H, 3·4, 3·5; As, 20·8; N, 3·8. $C_{14}H_{12}AsO_6N$ requires C, 46·0; H, 3·3; As, 20·5; N, 3·8%). It gave a red sodium salt.

The material soluble in cold bicarbonate solution was reprecipitated with hydrochloric acid. It consisted chiefly of azobenzene-4 : 4'-diarsonic acid (see Part I).

*4'-Hydroxy-3-nitrostilbene-4-arsonic Acid.*—4-Amino-2-nitrophenylarsonic acid (12·26 g.) was dissolved in a solution of sodium hydrogen carbonate (3·8 g.) in water (80 c.c.) to which was added sodium nitrite (3·6 g.) in water (10 c.c.). This solution was diazotised as above with 25% hydrochloric acid (32 c.c.). The clear solution was then poured into a solution of *p*-hydroxycinnamic acid (8·2 g.) in acetone (200 c.c.) to which sodium chloroacetate (17·5 g.) had been added. Gas evolution set in almost at once and became violent. Cupric chloride (5 g.) in water (10 c.c.) was added dropwise. The gas evolution lasted for approx. 30 minutes. A brownish precipitate separated which was filtered off, air-dried, digested with N-sodium hydroxide (50 c.c.), and filtered from the copper hydroxide. The alkaline solution was acidified with cold hydrochloric acid, and the precipitate filtered off, dried, and digested with a small amount of sodium hydrogen carbonate solution in which the greater part dissolved. 1·3 G. remained undissolved and were recrystallised from glacial acetic acid from which the *4'-hydroxy-3-nitrostilbene-4-arsonic acid* separated in reddish needles and rods which did not melt at 300° (Found: C, 45·9, 46·1; H, 3·5, 3·4; As, 20·7; N, 4·1%).

The 4-amino-2-nitrophenylarsonic acid was prepared by Dr. J. B. Polya, of the University of Tasmania, whom I wish to thank. I also thank Dr. K. F. Tettweiler, formerly of the University of Melbourne, for the microanalyses. This work was supported in part by a grant from the National Council of Health and Medical Research, Canberra.

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## 589. *A New Synthesis of Arsonic Acids. Part III.* Coupling of Furan, Thiophen, and β-2-Thienylacrylic Acid with Arsanilic Acid.*

By WALTER FREUND.

Furan, thiophen, and β-2-thienylacrylic acid have been coupled with diazotised arsanilic acid. They yield (2 ?)-*p*-arsonophenyl-furan and -thiophen respectively and (2 ?)-4'-arsonostyrylthiophen.

THE arsonic acids described in preceding Parts were prepared by treating diazotised arsanilic acid (in one case 4-amino-2-nitrophenylarsonic acid) with αβ-unsaturated carbonyl compounds. The reaction has now been extended to the synthesis of arsonic acids of the furan and thiophen series.

Johnson (*J.*, 1946, 895) coupled diazotised *p*-chloroaniline with furan according to Meerwein's method (*J. pr. Chem.*, 1939, **152**, 237), and obtained predominantly 2-*p*-chlorophenylfuran, with a small quantity of the 3-substituted furan. The reaction with *p*-arsanilic acid yielded only one compound which is probably 2-*p*-arsonophenylfuran. According to Gomberg and Bachmann (*J. Amer. Chem. Soc.*, 1924, **46**, 2339), thiophen and diazotised aniline yield only the 2-phenylthiophen, m. p. 42—43°, previously synthesised from β-benzoylpropionic acid and phosphorus pentasulphide by Kues and Paal (*Ber.*, 1887, **20**, 3141). On the other hand, Bamberger (*Ber.*, 1897, **30**, 370) obtained a compound of

* Part II, preceding paper.

m. p. 90—90·5° from nitrosoacetanilide and thiophen, and Möhlau and Berger (*Ber.*, 1893, **26**, 1994) reported a melting point of 56—57° for their product from benzenediazonium chloride, thiophen, and aluminium chloride. However, the melting point of Möhlau and Berger's compound could be raised by recrystallisation (see Bamberger, *loc. cit.*, footnote 3). The compound is considered to be 3-phenylthiophen. It is thus uncertain whether our product is 2- or 3-*p*-arsonophenylthiophen, though the former structure is preferred. β-2-Thienylacrylic acid and diazotised arsanilic acid give 2-4′-arsonostyrylthiophen in good yield; it thus differs from β-2-furylacrylic acid, which gives β-(5-*p*-arsonophenyl-2-furyl)acrylic acid as sole isolable product although 2-*p*-arsonophenyl-5-*p*-arsonostyrylfuran is doubtless also formed.

## Experimental

M. p.s are uncorrected.

2-p-*Arsonophenylfuran*.—Arsanilic acid (10·85 g.) dissolved in aqueous sodium hydroxide (2·1 g. in *ca.* 100 c.c.) was mixed with aqueous sodium nitrite (3·65 g. in 10 c.c.) and treated with 25% hydrochloric acid (32 g.) in the usual way. The clear solution was added to furan (3·4 g.) in acetone (100 c.c.) containing crystalline sodium acetate (*ca.* 40 g.). Gas evolution set in immediately and cupric chloride (10 g.) in water (*ca.* 20 c.c.) was added slowly. After 1 hour gas evolution ceased. The buff-coloured precipitate formed was filtered off, washed with acetone, dried, and dissolved in 2N-sodium hydroxide. The mixture was filtered and acidified, (2 ?)-p-*arsonophenylfuran* being precipitated as a brown solid. This was digested with cold acetone which on evaporation left a crystalline mass. Recrystallisations from alcohol gave needles or yellowish globuli, m. p. >300° in small yield (Found: C, 44·9, 44·9; H, 3·6, 3·5; As, 28·2. $C_{10}H_9O_4As$ requires C, 44·8; H, 3·4; As, 28·0%).

(2 ?)-o-*Arsonophenylthiophen*.—Arsanilic acid (10·85 g.) was diazotised as above and added to thiophen (4·2 g.) in acetone (100 c.c.) containing crystalline sodium acetate (35—40 g.). Cupric chloride (10 g.) in water (20 c.c.) was added dropwise with stirring during 1 hour and the solution was warmed on the water-bath to 20—22°, whereupon gas evolution set in. The *acid*, worked up as described above, crystallised from alcohol or glacial acetic acid as yellowish globuli, m. p. >300° (yield, 20—25%) (Found: C, 42·1, 42·1; H, 3·3, 3·4; As, 26·6. $C_{10}H_9O_3SAs$ requires C, 42·3; H, 3·2; As, 26·4%).

β-(5-p-*Arsonophenyl-2-furyl)acrylic Acid*.—Arsanilic acid (10·85 g.) was diazotised as above and added to a solution of furylacrylic acid (6·9 g.) in acetone (120 c.c.) in which crystalline sodium acetate (35—40 g.) was suspended. Gas evolution commenced immediately and became violent even without the addition of cupric chloride. The temperature which after the addition of the diazonium solution was approx. 8—10° rose to 24—25° and cooling was advisable. Addition of cupric chloride solution is advisable as the copper salt of the arsonic acid formed is easily separated. The solid was worked up as above. The dry *arsonic acid* was extracted with absolute alcohol which on evaporation deposited small not very well-developed crystals, m. p. >300° (Found: C, 46·1, 46·0; H, 3·4, 3·4; As, 22·3. $C_{13}H_{11}AsO_6$ requires C, 46·2; H, 3·3; As, 22·2%). The major part of the hydrochloric acid precipitate (insoluble in cold alcohol), probably the 2-*p*-arsonophenyl-5-4′-arsonostyrylfuran, could not be obtained free from nitrogen as it could not be recrystallised.

2-p-*Arsonostyrylthiophen*.—An attempt to prepare thienylacrylic acid from thiophen-2-aldehyde by a Perkin condensation according to Biedermann (*Ber.*, 1886, **19**, 1855) gave a very poor yield, most of the aldehyde being resinified. A Doebner condensation with malonic acid (approx. 2 mols.) gave a 75—80% yield. After recrystallisation from water the β-2-thienylacrylic acid, m. p. 144—145° (Cohn, *Z. physiol. Chem.*, 1893, **17**, 283, gives this m. p.).

Arsanilic acid (10·85 g.) was diazotised as above and added to a solution of β-2-thienylacrylic acid (7·7 g.) in acetone (125 c.c.) in which crystalline sodium acetate (*ca.* 40 g.) was suspended. After addition of a few drops of aqueous cupric chloride (10 g. in 20 c.c.), gas evolution set in and a buff-coloured precipitate began to form. At 20—22° gas evolution was considerable and lasted for approx. 1 hour during which all of the cupric chloride solution was added. After a few hours the precipitate was collected (from the solution 1·3 g. of unchanged thienylacrylic acid were recovered). The solid was dissolved in 2N-sodium hydroxide, which was filtered and acidified. The precipitated *arsonic acid*, a brown solid, was dried (yield, 8·8 g.) and twice recrystallised from acetic acid (orange-red solution), forming plates and prisms, m. p. >300° (yield, 30%) (Found: C, 46·3, 46·3; H, 3·8, 3·8; As, 22·1, 22·3; S, 10·1, 10·0. $C_{12}H_{11}O_3SAs$ requires C, 46·5; H, 3·6; As. 24·2; S, 10·3%).

All analyses (except that of 2-*p*-arsonostyrylthiophen) were by Dr. K. F. Tettweiler, formerly of the University of Melbourne, whom I wish to thank. This work was supported in part by a grant from the National Council of Health and Medical Research, Canberra.

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## 590. *The Action of Denaturing Agents on Deoxyribonucleic Acid.*

By B. E. CONWAY AND J. A. V. BUTLER.

Phenol or urea causes a considerable decrease in the viscosity of solutions of deoxyribonucleic acid, and the effect is irreversible in that the original viscosity is not recovered when the agent is removed by dialysis. Measurements of viscosity, diffusion, and sedimentation indicate that the interaction between the particles is diminished by the presence of the agent, and that when the agent is removed the particles assume a more compact form. It is suggested that this is due to the breakage of the hydrogen bonds to which the configuration of the nucleic acid particles is due.

THAT urea and other effective protein denaturants reduce the viscosity of solutions of deoxyribonucleic acid was noted by Jenrette and Greenstein (*Cold Spring Harbor Symp.*, 1941, **9**, 236). We have re-examined this phenomenon since it was hoped that it would elucidate the loss of viscosity produced by substances of the " mustard " class at much smaller concentrations (Butler and Smith, *J.*, 1950, 3411), and also provide information on the properties of the nucleic acid. Fig. 1 shows effects of applied pressure on the viscosity of nucleic acid solutions containing urea, as determined in a capillary viscometer. Similar effects are produced by phenol (Fig. 2) which is also known to act as a protein denaturant (Alexander, *Ann. New York Acad. Sci.*, 1951, **53**, 24). The effect of phenol is mainly on the low-pressure viscosity, *i.e.*, the increased viscosity which is observed at low rates of flow is greatly diminished, but the viscosity at higher pressures is not greatly affected. The effect of concentration of the added substance at a constant pressure is shown in Fig. 3. It can be seen that phenol is more effective than urea for a given molar concentration, but the phenomena observed are of course limited by the solubility. Ethanol also reduced the viscosity somewhat, but its main effect is at small concentrations ($<0{\cdot}2$M)—increasing the concentration from 0·2 to 2M has very little further effect.

It has been found that the effects of both urea and phenol on the nucleic acid are irreversible, *i.e.*, the viscosity is not restored by removing the solute by dialysis. In fact the following table shows that the viscosity of the solutions is decreased further when the agent is removed. Only small changes are brought about by dialysis and freeze-drying of nucleic acid solutions in neutral water.

| | $\eta_{rel.}$ at 60 mm. |
|---|---|
| (1) 0·1% Solution of nucleic acid I | 14·0 |
| (2) ,, +7% of phenol | 10·3 |
| (3) As (2) but after dialysis and freeze-drying | 4·9 |
| (4) 0·1% Solution of nucleic acid II | 10·5 |
| (5) ,, after dialysis | 10·0 |
| (6) ,, after dialysis and freeze-drying | 9·2 |

As is well known, the viscosity of nucleic acid solutions is decreased by small concentrations of salt. This has been stated to be reversible (Greenstein and Jenrette, *J. Nat. Cancer Inst.*, 1940, **1**, 77). It has been found that, in the presence of urea and phenol, the addition of an electrolyte has very little effect on the viscosity (Fig. 4), *i.e.*, urea or phenol produces an effect similar to that of a small concentration of electrolyte. When the phenol or urea is removed by dialysis, the viscosity is again reduced by the addition of salts.

The fact that phenol and urea produce permanent effects on the nucleic acid suggests that their action is similar to the denaturation of proteins. It has been found (cf. Neurath and Saum, *J. Biol. Chem.*, 1939, **128**, 347) that denaturing agents disorganise the configuration of the native protein molecule, probably by the breakage of the hydrogen bonds

(Mirsky and Pauling, *Proc. Nat. Acad. Sci.*, 1936, **22**, 439; Cannan, Kilpatrick, and Palmer, *Ann. New York Acad. Sci.*, 1941, **41**, 2437) which maintain the protein in its original configuration. This is supported, for example, by infra-red absorption spectra (Buswell, Krebs, and Rodebush, *J. Phys. Chem.*, 1940, **44**, 1126; Richards and Thompson, *J.*, 1947, 1248). Denaturing agents in general appear to be substances which are capable of breaking such bonds. This view is supported by previous observations of the action of acids and alkalis on deoxyribonucleic acid (Creeth, Gulland, and Jordan, *J.*, 1947, 1141; Zamenhof and Chargaff, *J. Biol. Chem.*, 1950, **186**, 207) which has been described as denaturation. It was also shown by Gulland, Jordan, and Taylor (*J.*, 1947, 1131) that, when the nucleic acid is titrated with acid or alkali, hysteresis is observed—the titration curves starting from the neutral region differ from those obtained on back-titration from acid or alkaline pH—which was attributed to the titratable amino- and hydroxyl groups' being united in hydrogen bonds and inaccessible to titration until fairly acid or alkaline pH is reached. Chargaff (*J. Cell. Comp. Physiol.*, 1951, **38**, Suppl. 1, 41; *Fed. Proc.*, 1951, **10**, 654)

FIG. 1. FIG. 2.

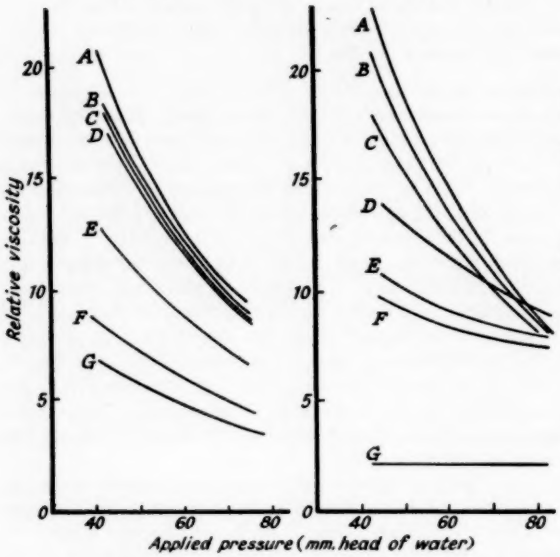



FIG. 1. *Effect of urea on the viscosities of 0·1% deoxyribonucleic acid.*

*A, Nucleic acid in water; B—G, nucleic acid in urea solutions, viz.: B,* $10^{-3}$M; *C,* $10^{-2}$M; *D,* 0·1M; *E,* M; *F,* 4·0M; *G,* 7·0M.

FIG. 2. *Effect of phenol on the viscosities of 0·1% deoxyribonucleic acid solutions.*

*A, Nucleic acid in water; B—F, nucleic acid in phenol solutions, viz.: B,* 0·1%; *C,* 0·5%; *D,* 2·0%; *E,* 5·0%; *F,* 7·0%; *G, nucleic acid in* 7·0% *phenol and* 0·1N*-sodium chloride.*

have shown that in all the deoxyribonucleic acids investigated by them there is a fairly constant balance between primary amino-groups and hydroxyl groups, so that the nucleic acid may be regarded as an organised structure maintained by intra-molecular hydrogen bonds. The effects of reagents such as phenol and urea which break hydrogen bonds may thus be akin to denaturation processes, such as occur with native proteins.

We have endeavoured to characterize the changes brought about by these agents by a study of the viscosity and of other properties in solution.

*Viscosities of Nucleic Acid Solutions.*—Fig. 5 shows the variation of the reduced viscosity $(\eta - \eta_0)/c\eta_0$ with the concentration of the nucleic acid at a constant shear rate (250 sec.$^{-1}$) under the conditions stated in the legend. For aqueous solutions of untreated nucleic acid over the concentration range studied, the reduced viscosity decreases with increasing concentration, except in 0·1N-sodium chloride where there is a slight trend in the opposite direction. The diminution is much less than that observed with certain flexible polyelectrolytes (Fuoss and Strauss, *Ann. New York Acad. Sci.*, 1949, **41**, 836) and is possibly due to the tendency of elongated molecules to arrange themselves with their long axes in the same direction and, to some extent, to the hydrodynamic conditions of flow of viscous fluids in a capillary tube.

The viscosities at the concentrations in question are largely determined, in the absence of salt, by intermolecular interactions (cf. Butler and James, *Nature*, 1951, **167**, 844). It has been shown by Pouyet (*J. Chim. phys.*, 1951, **48**, 90) and Benoit (*ibid.*, p. 86) that these interactions persist in nucleic acid solutions to concentrations of the order of 0·001%; and, although they are diminished by salts, they probably still occur to some extent in the

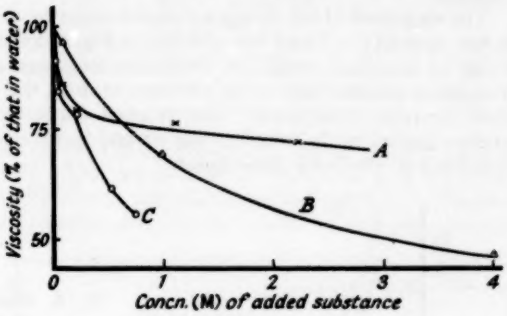



FIG. 3. *Effect of concentration of the added substances on the viscosity of nucleic acid.*

*A, Ethanol.*
*B, Urea.*
*C, Phenol.*

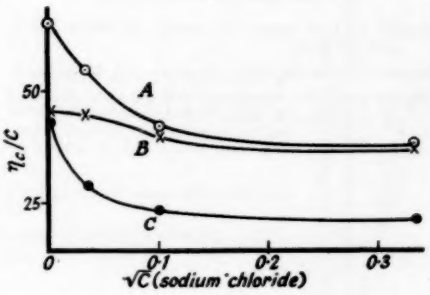



FIG. 4. *Effect of sodium chloride concentration on reduced viscosity of deoxyribonucleic acid (0·05%) at constant shear rate (250 sec.$^{-1}$).*

*A, Nucleic acid alone.*
*B, Nucleic acid in 6M-urea.*
*C, Nucleic acid after treatment wth 6M-urea, followed by dialysis.*

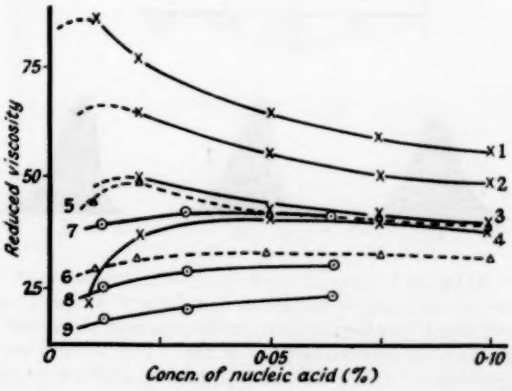



FIG. 5. *Specific viscosities of deoxyribonucleic acid solutions at a constant rate of flow.*

*In water:* 1, *water only;* 2, 0·001N-NaCl; 3, 0·01N-NaCl; 4, 0·1N-NaCl.

*In 6M-urea:* 5, *6M-urea only;* 6, *6M-urea* + 0·1N-NaCl.

*In water after treatment with 6M-urea and dialysis:* 7, *water only;* 8, 0·001N-NaCl; 9, 0·1N-NaCl.

salt solutions used by us. The reduced viscosities found are therefore considerably greater than those at zero concentration. It is impossible in these circumstances to measure, with a capillary viscometer, solutions which are sufficiently dilute to have intrinsic viscosities approaching those at zero concentration; the information about molecular shape and dimensions which can be obtained from our measurements is thus limited. It can be seen, however (Fig. 5), that the effect of urea on the viscosity–concentration curve at constant rate of flow is very similar to that of sodium chloride and we may conclude that its presence mainly influences the interaction of the particles. After treatment with urea followed by

dialysis, the sensitivity of the material to salt concentration is similar to that of the untreated nucleic acid; by a comparison with the original nucleic acid, this must be ascribed to a diminution of interactions between the particles, produced by the salt, but it is evident that the particles have also been permanently modified in some way during the removal of the urea. Replacement of the urea after its removal does not restore the viscosity.

The character of the changes brought about by urea is shown by the effect of rate of flow on the viscosity. These are plotted in Fig. 6 for a constant nucleic acid concentration. It can be seen that, while the viscosities measured are a function of the rate of flow both in aqueous solution and in the presence of urea, the dependence on flow rate is largely lost when the urea is removed. The simplest explanation is that the particle becomes more symmetrical when the urea is removed, and this is supported by diffusion and sedimentation observations which are given below.

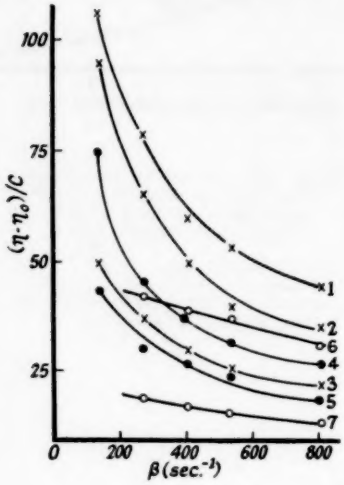



FIG. 6. *Reduced viscosities of nucleic acids* (0·02%) *as a function of the rate of flow.*

*Nucleic acid in water:* **1**, *alone;* **2**, 0·01N-NaCl; **3**, 0·1N-NaCl.

*Nucleic acid in* 6M-*urea:* **4**, *alone;* **5**, 0·1N-NaCl.

*Nucleic acid after treatment with* 6M-*urea, followed by dialysis:* **6**, *water;* **7**, 0·1N-NaCl.

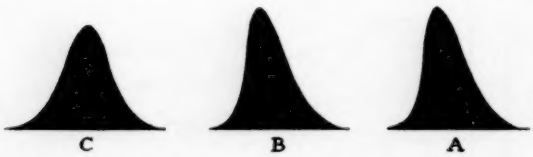



FIG. 7. *Schlieren diagrams of boundary in diffusion of nucleic acid* (0·2%). *Diffusion is occurring from right to left.*

*A, In* 0·1N-NaCl.
*B, In* 6M-*urea* + 0·1N-NaCl.
*C, In* 0·1N-NaCl, *after treatment with* 6M-*urea, followed by dialysis.*

*Sedimentation and Diffusion.*—Measurements of sedimentation and diffusion constants have been made of nucleic acid in water, 6M-urea, and 7% phenol solution, and of nucleic acid which has been treated with urea and recovered by dialysis in water, in all cases in the presence of 0·1N-sodium chloride. The results are shown in the annexed Table. The significance of the diffusion values is subject to considerable uncertainty owing to the asymmetric nature of the boundary curves (cf. Butler and James, *Nature*, 1951, **167**, 844). The asymmetry remains in the presence of phenol and of urea (Fig. 7); but the boundaries obtained with the substance which has been treated with urea and dialysed are quite symmetrical. In the sedimentation, the sharp boundary characteristic of nucleic acid is maintained in the presence of urea and phenol, but in the dialysed material a broader band is observed which spreads more rapidly than is to be expected from the diffusion constant. This is evidence of the heterogeneity of this material with respect to sedimentation constant. From the figures (Table) it is seen that on the whole the values obtained in the presence of phenol and urea are not greatly different from those in water. On the other hand the

*Sedimentation (S) and diffusion (D) constants of nucleic acid, in the presence of* 0·1N*-sodium chloride.*

| Solution | | Concn. of nucleic acid, % 0·05 | 0·1 | 0·2 | *M* | $f/f_0$ | ρ |
|---|---|---|---|---|---|---|---|
| Water ..... | *S* | 9·9 | 7·4 | 5·8 | — | — | — |
| | *D* | 4·65 | 4·00 | 4·05 | $1·5 \times 10^6$ | 6·9 | 316 |
| Aq. phenol (7%) ..... | *S* | 11·1 | 8·75 | 5·4 | — | — | — |
| | *D* | — | 5·1 | 3·7 | — | — | — |
| 6M-Urea ..... | *S* | 8·5 | 6·5 | 6·2 | — | — | — |
| | *D* | — | — | — | — | — | — |
| ,, after dialysis | *S* | 14·7 | 11·6 | — | — | — | — |
| | *D* | 9·1 | 6·3 | — | $7·5 \times 10^5$ | 3·35 | 50 |

nucleic acid which has been treated with 6M-urea and dialysed has values of both *S* and *D* greater than those of the nucleic acid itself. This means that the molecular weight is of the same order in the two cases, but that the frictional resistance to sedimentation or diffusion, which will occur if the molecule is more compact, is less in the latter. The Table gives the

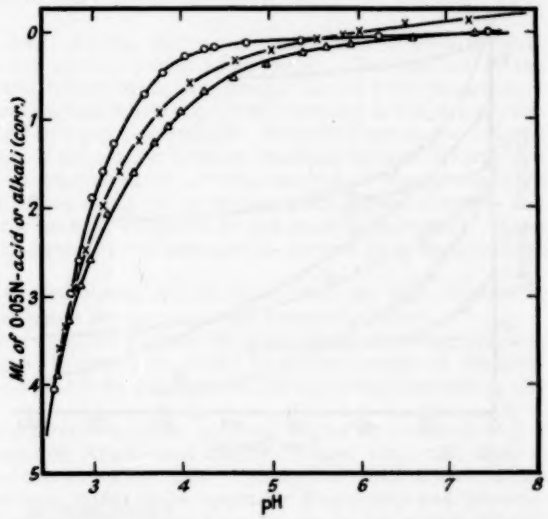



FIG. 8. *Titration curves of nucleic acid.*

○ *Forward-titration from* pH 7·5 *of untreated nucleic acid, and of nucleic acid treated with* 6M*-urea and dialysed.*

× *Back-titration of urea-treated nucleic acid.*

△ *Back-titration of untreated nucleic acid.*

molecular weights *M*, frictional coefficient $f/f_0$, and axial ratios ρ calculated from extrapolation of *S* and *D* to zero concentration. These are subject to considerable uncertainty for the reasons mentioned above, but they confirm the viscosity measurements by indicating a more compact molecule after treatment with urea and its subsequent removal.

*Titration Curves.*—As mentioned above, Gulland, Jordan, and Taylor (*loc. cit.*) regarded the hysteresis which they observed in the titration curves of the nucleic acid as evidence that hydrogen bonds were broken at extreme pH's. If phenol and urea break the hydrogen bonds the titration curves after such treatments should resemble the back-titration curve after treatment with acid or alkali. Experiments were made with the nucleic acid, after treatment with urea (6M) and removal of the urea by dialysis. On the alkaline side the results were vitiated by a small amount of urea remaining after dialysis (see below) which appeared to be hydrolysed to ammonium carbonate during the titration and gave rise to unsteady pH values. On the acid side no interference by urea was observed, and the forward- and back-titration curves of untreated and treated nucleic acid are shown in Fig. 8.

The amount of hysteresis observed in the urea-treated nucleic acid is appreciably less than that in the untreated material. This is similar to the effect of ultrasonic irradiation observed by Lee and Peacock (*J.*, 1951, 3374). However, the *forward*-titration curve is

identical in the two cases, which suggests that there is no effective difference in the titratable groups in the forward-titration, although the back-titrations differ somewhat. This must mean that the amino- and hydroxyl groups of the urea-treated nucleic acid are still inaccessible to titration by acid until fairly low pH's are reached; *i.e.*, if the original interpretation of the hysteresis is correct, they are still hydrogen-bonded in the urea-treated material. This indicates that the hydrogen bonds have been reconstituted although not necessarily between the same groups.

*Effects of Small Concentrations of the Denaturing Agents.*—If the effects of urea and phenol are denaturing processes involving the breakage of hydrogen bonds, they might be expected to occupy a finite time, as is usually the case in such observations as can be made with proteins. With the higher concentrations of urea and phenol, as used above, the viscosity is immediately affected by the addition, and then remains nearly constant. It was found, however, that with small concentrations of these agents, the initial change is small and is followed by a slow change of viscosity which leads eventually to values of the same order as those reached immediately in the presence of higher concentrations (Fig. 9).

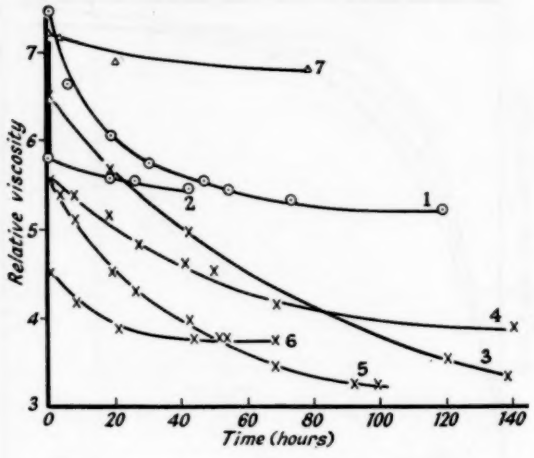



FIG. 9. *Effects of various concentrations of phenol and urea on 0·07% aq. sodium thymonucleate at* $\beta = 250$ *sec.*$^{-1}$.

1, 0·05M-*Phenol*. 2, 0·5M-*Phenol*.
3, 0·05M-*Urea*. 4, 0·15M-*Urea*.
5, 0·5M-*Urea*. 6, 6M-*Urea*.
7, *Control*.

## EXPERIMENTAL

*Materials.*—Samples of deoxyribonucleic acid were prepared by the methods previously described (*J.*, 1950, 3411). Most of the present work was done with preparations from bovine thymus gland, but a few observations were made with a preparation from herring sperm which had similar sedimentation and viscosity constants. The preparations were all dialysed for a week or more with constant changes of distilled water and the acids were then precipitated with ethanol and dried.

*Viscosity, Sedimentation, and Diffusion Measurements.*—Viscosities were determined at various heads of solution in viscometers of the Frampton type. The mean shear rates $\beta$ were calculated by Kroepolin's formula, $\beta = 8V/3\pi r^3 t$, where $V$ is the volume (ml.) of liquid flowing in time $t$ (sec.) through the capillary of radius $r$ (cm.) (*Kolloid Z.*, 1929, **47**, 294).

The salt solutions were made up in the viscometer by inserting ground-glass weighing bottles containing the salt into a corresponding ground socket on the viscometer.

Sedimentation rates were determined with the Spinco ultra-centrifuge at approx. 60,000 r.p.m.

Diffusion rates were determined by observing the boundary between the solution and the corresponding solvent at different times by means of the schlieren optical system in a Perkin–Elmer electrophoresis apparatus.

*Electrometric Titrations.*—Aliquots (25 ml.) of 0·2% deoxyribonucleic acid solutions in 0·1N-potassium chloride, free from carbon dioxide, were titrated in a closed flask, with a glass

electrode which gave a linear pH response up to pH 13. The solutions were stirred by nitrogen throughout the titrations. In the acid direction the solutions were titrated with 0·05N-hydrochloric acid and in the alkaline direction with 0·05N-potassium hydroxide (carbonate-free). Corrections for water (Gulland, Jordan, and Taylor, *J.*, 1947, 1131) were determined by titrating an equal volume of 0·1N-potassium chloride from pH 7 to 2·5 and 11·5 respectively, under conditions similar to those used for the nucleic acid titrations.

In experiments with urea, recrystallized urea was added to the nucleic acid solutions to give a concentration of 6M. After 3 hours the mixture was dialysed in a rocking dialyser against a large volume of distilled water, which was changed six times. Air was excluded in this dialysis and all the water used had been previously boiled out and cooled in nitrogen.

*Determination of Urea retained by the Nucleic Acid.*—Since the urea produces irreversible effects it was desirable to determine the amount remaining after dialysis. The solution was depolymerized with deoxyribonuclease to release urea trapped in the polymerized form and was concentrated at 30° *in vacuo*. The urea present was then determined as ammonia by addition of urease, followed by potassium carbonate, in a Conway microdiffusion unit. A similar control experiment was made with a solution containing urea only. The concentration found in the nucleic acid solution was $4 \times 10^{-4}$M and that in the control $1 \times 10^{-4}$M. The difference is equivalent to 1 molecule of urea bound to every 20—25 nucleotide units. We are indebted to Miss E. M. Press for these analyses.

*Solubility of Urea-treated Nucleic Acid.*—During dialysis of nucleic acid in 6M-urea some material was always precipitated, but the amount varied considerably. The amount of the precipitate, determined by weighing after freeze-drying, was greater from a 0·4% than from a 0·1% nucleic acid solution, but the fraction of the whole precipitated was less in the former case. On one occasion, 15—20% of the nucleic acid was precipitated. When still wet in the solution the precipitates are soluble in 6M-urea, but after being dried are insoluble in both 6M-urea and 30% lithium bromide solution. On concentration of a 0·1% solution of the urea-treated nucleic acid (soluble part) to 0·4% at 35° *in vacuo*, no immediate precipitation occurred, but during 24 hours the solution deposited a gel-like precipitate on the walls of the vessel. When separated, this material is insoluble in water but is dispersed in 6M-urea to a fairly viscous opalescent solution.

When a dialysed solution of urea-treated nucleic acid is freeze-dried, the solid obtained is insoluble in water. It swells considerably but always remains as a separate phase.

These observations suggest that the precipitates obtained on removal of urea are aggregations formed by intermolecular formation of hydrogen bonds, which have been broken by the urea. The solubilization of the precipitates by urea will be due to disassociation of the aggregate by the further breakage of such bonds.

*Effect of Salts on Nucleic Acid.*—As lithium bromide has been stated to be effective as a hydrogen-bond breaker (Ambrose, Bamford, Elliott, and Hanby, *Nature*, 1951, **167**, 264), it was of interest to examine the effect of this and other salts. The normal effect of salts on the viscosity of nucleic acid solutions has been stated to be reversible (Greenstein and Jenrette, *loc. cit.*). A considerable measure of reversibility follows from the use of 2M-sodium chloride during the preparation. We have found, however, that complete recovery of the viscosity does not always occur after treatment with sodium chloride, followed by its removal by dialysis. The following table gives characteristic values. With sodium chloride a fairly good recovery is

*Relative viscosities of nucleic acids.*

| Added salt : | NaCl (0·1N) * | LiBr (6N) † | NaCl (N) † | NaCl (0·1N) ‡ |
|---|---|---|---|---|
| (1) In water ..... | 17·8 | 6·3 | 6·3 | 9·4 |
| (2) In salt solution ..... | 3·3 | 1·7 | 2·1 | 4·4 |
| (3) As (2), but dialysed ... | 10·5 | 1·1 | 5·7 | 8·0 |

* Thymonucleic acid, 0·1%. † Thymonucleic acid, 0·07%. ‡ Herring sperm nucleic acid, 0·1%.

obtained with samples having a low initial viscosity, but not when the initial viscosity is high. With lithium bromide (6M) no recovery of the viscosity occurs. It appears from this that salts may bring about some permanent changes in the nucleic acid, but only in the case of the lithium salt does the effect approach that of urea and phenol. The great effect of salts on the viscosity is probably due to a decrease of the interactions between the nucleic acid particles. It is evident that, when interrupted, the interactions may not be fully restored by removal of the salt and that some change of configuration may occur. This is particularly marked with the more highly viscous samples of nucleic acid. This subject is being studied further.

## Discussion

It has been shown that at high concentrations urea and phenol have a considerable effect on the viscosity of the nucleic acid and that this effect is irreversible in that the initial state is not restored when the added substance is removed. Detailed studies in the case of urea have shown that the effect observed in the presence of 6M-urea is similar to that produced by 0·1M-salts and is to be attributed for the most part to a decrease of the interaction of the particles, without it being necessary to postulate any great change of form of the particles themselves. However, when urea is removed by dialysis a permanent modification occurs, and observations of diffusion and sedimentation suggest that the particles assume a more compact form. Urea and phenol are well known to act as hydrogen-bond " breakers " and it is by virtue of this property that they denature proteins. It is probable that they do this by taking the place of either partner of the groups united by hydrogen bonds, which determine the configuration.

Urea molecules can no doubt form hydrogen bonds with each other, and in high concentrations it is likely that such associations occur. So long as a high concentration of urea is present, we can account for the absence of any significant change of configuration, although some of the configurational bonds may be broken, if the molecule is maintained in its original configuration by groups of urea molecules uniting the parts. When, however, the excess of urea is removed by dialysis, the particle of nucleic acid will be able to take up a new and more compact configuration. Phenol may be able to function similarly owing to the formation of complex molecules under the influence of the van der Waals attraction of the aromatic rings.

It seems unlikely that the amount of phenol or urea remaining after dialysis is sufficient to produce by itself a marked change of the behaviour of the nucleic acid. It would be sufficient to explain the observed effects, if the removal of the agent attached to some of the previously bonded amino- or hydroxyl groups permits rotation or bending of the particle, so that the latter takes up another configuration in which hydrogen bonds are re-formed, possibly with other partners.

That both urea and phenol when present in small concentrations produce similar effects, but at a very slow rate, also shows that the effect of these agents is not purely physical, such as might be produced by changing the environment of the nucleic acid particles (*e.g.*, by change of dielectric constant) but is due to a reaction which occurs at a finite rate. It is evident from the results that the final viscosities obtained with urea, at all the concentrations studied, are lower than those observed with *any* concentration of phenol. This suggests that urea brings about changes in the nucleic acid particles which are not effected by phenol, and this indicates that the hydrogen bonds concerned in these changes are not all of the same kind (cf. Cavalieri and Angelos, *J. Amer. Chem. Soc.*, 1950, **72**, 4686). Further experiments will be required to establish these conclusions fully but, since it is desirable to work at concentrations at which the nucleic acid particles do not interact with each other, it will be necessary to use experimental methods which are applicable at very low concentrations.

This work was communicated (in part) to the Protein and Nucleic Acid Conference held at New Hampton, N.H., in August 1951.

We are indebted to Mr. P. G. Avis for technical assistance, to Miss E. M. Press for analyses, and to Mr. D. W. F. James for help with the ultra-centrifuge. This investigation has been supported by grants to the Royal Cancer Hospital and Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

Chester Beatty Research Institute,
Royal Cancer Hospital, London, S.W.3. [*Received, February 19th*, 1952.]

## 591. *The Phosphorescence Emission of Benzophenone.*

By J. Ferguson and H. J. Tinson.

The phosphorescence emission of benzophenone has been examined and recorded. The emission was found to be of two different types, the proportion of each depending on the concentration of benzophenone. An attempt has been made to explain this dependency by consideration of intermolecular migration of excitation energy.

That the phosphorescence emission observed from many organic molecules is a transition from a triplet to the ground singlet state of the molecule was suggested by Lewis and Kasha (*J. Amer. Chem. Soc.*, 1945, **67**, 994; 1944, **66**, 2100). Before this work very little was known about such states and no systematic investigation of them had been attempted. The field has been reviewed by Kasha (*Chem. Reviews*, 1947, **41**, 401). Since then, however, further work has been published by a group of American workers and a number of Russian authors.

The phosphorescence emission of benzene has been analysed by Shull (*J. Chem. Phys.*, 1949, **17**, 295) and the perturbing singlet claimed to be of symmetry $^1B_{1u}$. Much stronger spectroscopic evidence, however, points to this level as having the symmetry $^1B_{2u}$. McClure (*ibid.*, p. 905) has measured the lifetimes of the emissions of a number of molecules. He has attempted to calculate theoretically these lifetimes, but finds them to be in bad agreement with experiment. McClure has also derived selection rules for the mixing of singlet and triplet states (*ibid.*, p. 665), but their validity has yet to be proved.

These workers have assumed that the mechanism of population of the triplet state is that given by Kasha (*loc. cit.*), *i.e.*, a spin-orbit interaction operator controls an internal conversion between singlet and triplet states. Our observations with benzophenone, however, show evidence for a mechanism by means of which the triplet state is populated by intermolecular migration of excitation energy.

The phosphorescence emission of benzophenone was photographed, solutions in light petroleum which ranged from $10^{-2}$ to $10^{-4}$M being used. The resulting tracings are shown in Figs. 1, 2, and 3. As can be seen, the spectrum is changing between these two values. At low concentrations the emission appears blue when viewed through the phosphoroscope but becomes progressively green as the concentration is increased. As the blue emission has the shorter life, a photograph of the green emission was obtained by revolving the phosphoroscope by hand and using a solution which had a predominantly green emission, *i.e.*, of concentration about $10^{-2}$M. The tracing of the green emission is shown in Fig. 4. Although the green emission is present at low concentrations it is so weak that the tracing in Fig. 1 was taken to represent the blue emission. The positions of the peaks in the blue and the green emissions are shown in the table.

| Positions of peaks, in Å | | | | |
|---|---|---|---|---|
| Blue emission | | Green emission | | |
| 4190 | 5310 | 4480 | 5060 | 5360 |
| 4500 | 5700 | 4600 | 5120 | 5500 |
| 4860 | | 4780 | 5280 | 5660 |
| | | 4900 | | |

Only a preliminary attempt was made to compare frequency differences with known Raman frequencies. A favourable comparison was obtained with both emissions, but the accuracy of measurement is low and so little is known about the vibrational modes of a molecule the size of benzophenone that the subject was not pursued further.

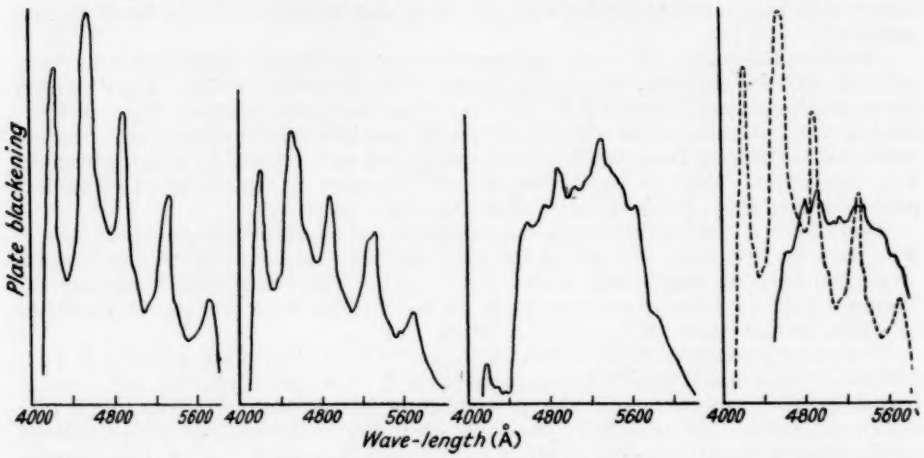
There seems to be no doubt that the blue emission corresponds to that obtained by Lewis and Kasha (*loc. cit.*) with the exception that we have obtained a fifth band at longer wave-lengths which they did not report.

Visual scanning of the spectrum by means of a monochromator gives an "excitation

spectrum " different from that observed with other compounds not allied to benzophenone, *e.g.*, aromatic hydrocarbons, amines, etc. In these cases the excitation spectra are similar to those usually found, owing to the phenomenon of internal conversion, in fluorescence (Pringsheim, " Fluorescence and Phosphorescence," Interscience Publ., New York, 1949, p. 306). The excitation spectrum of benzophenone, however, has two maxima corresponding to the two regions of absorption lying above 2300 Å. This suggests that internal conversion between the first two singlets is a process of low probability and the two singlets differ in character. The first region of absorption has been shown by McMurray (*J. Chem. Phys.*, 1941, **9**, 231) to be of the type $N–E_1$, involving a non-bonding electron from the oxygen atom, and the second of the type $N–V_1$ peculiar to the aromatic ring system.

The effect of change in concentration on the relative intensities of the phosphorescence excited in the two regions is most noticeable; that excited in the first region of absorption increasing greatly in intensity with increase in concentration.

FIG. 1. FIG. 2. FIG. 3. FIG. 4.




*Benzophenone at the concentration of* 1·2 × $10^{-4}$M. *Benzophenone at the concentration of* 9·3 × $10^{-4}$M. *Benzophenone at the concentration of* 9·5 × $10^{-3}$M. *The two phosphorescences of benzophenone.*

Behaviour similar to that of benzophenone was also observed with allied substances such as acetophenone, benzoin, and benzil.

Benzophenone, then, as a representative of a class of compounds is unique in that phosphorescence from two different triplet levels is observed. This is because internal conversion is a process of low probability. Although there is no proof of the nature of these levels, we have assumed them to be triplet in accordance with the ideas of Lewis and Kasha (*loc. cit.*). It remains then to discuss the mechanism of population of these levels. At all the concentrations we have employed both emissions are present in varying degrees, although at concentrations of the order $10^{-4}$M the glow has only a very faint trace of green.

The change in nature of the emission is most noticeable between $10^{-3}$ and $10^{-4}$M-concentration. This comparatively abrupt change suggests energy transfer. Franck and Livingston (*Rev. Mod. Physics*, 1949, **21**, 505) state that a molecule, excited by light of wave-length shorter than that absorbed by a second compound which is also present in the system, should be able to transfer its energy to a molecule of the latter type. The efficiency of this transfer should be high if the fluorescence spectrum of the primarily excited molecule overlaps the absorption spectrum of the second molecule.

Förster (*Ann. Physik*, 1948, **2**, 55) has approached the problem of energy exchange and fluorescence by means of quantum mechanics and has obtained expressions for the critical

distance between similar molecules below which exchange can occur. He has considered only exchange between similar molecules in the same region of the spectrum.

If, in a system containing the one species of molecule, the fluorescence spectrum of the first singlet overlaps the absorption spectrum of the first (or higher) triplet, then energy transfer should be possible between molecules excited in the lowest singlet level (through internal conversion or direct transition from the ground state) and unexcited molecules lying within the critical region. The latter, on being transferred to the lowest triplet level, would be capable of returning to the ground state and emitting their characteristic phosphorescence.

We consider that the above explanation can be applied to the emission observed with benzophenone. It is the general rule that the difference in energy between the lowest singlet and triplet is low enough for the fluorescence spectrum of the former to overlap the absorption spectrum of the latter.

The possibility of energy migration in phosphorescence has been considered by Weissman (*J. Chem. Phys.*, 1950, **18**, 1258) in a study of various metal complexes. He dismissed this, however, when he found no appreciable change in the degree of polarisation when the concentration was changed from $10^{-4}$ to $10^{-6}$M. The range of concentrations he used, however, was very limited and we feel that a more thorough investigation was needed to reach the conclusion of no energy transfer.

We do not as yet know the extent to which migration of energy populates the triplet states of other molecules, but a preliminary observation with the benzene phosphorescence shows that energy can definitely be transferred. This is shown by the quenching of the phosphorescence of benzene by the presence of iodobenzene, which does not have a phosphorescence emission (unpublished work).

We suggest that the difficulties so far experienced in attempted theoretical work may lie in the neglect of energy transfer. Work is proceeding along these lines.

## Experimental

*Benzophenone.*—A B.D.H. sample, recrystallised from alcohol, then light petroleum, and three times sublimed, had m. p. 49°.

*Light Petroleum.*—This solvent was used for the work as it sets to a transparent glass at the temperature of boiling nitrogen. It was purified by treatment with 20% oleum until the acid layer was no longer coloured, washed with sodium hydroxide solution and then water, dried, and distilled; it boiled at 58—63°/760 mm. and then had no phosphorescence emission, this being the criterion of purity.

*Apparatus.*—The method and apparatus for recording the phosphorescence emissions were essentially as used by Lewis and Kasha (*loc. cit.*). A Pyrex Dewar vessel with Corex windows, of short-wave transmission limit about 2300 Å, and, instead of a rotating can, two discs, with four holes in each staggered in relation to one another rotating at about 1800 r.p.m. were employed.

The spectrograph was a Hilger F4 high-aperture instrument with non-linear dispersion. All photographs were taken on thin glass panchromatic super XX Kodak plates. The slit width used in all cases was 0·2 mm. with exposures of 10 and 30 minutes. The spectrogram densities were determined with a Baird Associates non-recording densitometer. A Hanovia mercury ultra-violet lamp was used as a source of exciting radiation.

We thank Dr. T. Iredale for his interest and helpful discussions of this work, and Mr. T. M. Dunn for the design of the Corex-windowed Dewar flask.

University of Sydney, Australia. [*Received, March 17th*, 1952.]

## 592. *The Nitration of Guanidine in Sulphuric Acid. Part I. The Reversible Conversion of Guanidine Nitrate into Nitroguanidine.*

By R. J. J. Simkins and Gwyn Williams.

The conversion of guanidine nitrate into nitroguanidine in sulphuric acid–water media is reversible. Equilibrium is approached at a measurable rate, from either side, in 71—83% sulphuric acid, very rapidly in media containing more than 85% sulphuric acid. On the supposition that the effective nitrating agent is the nitronium ion in all the media considered, mass-action considerations predict that conversion into nitroguanidine should be most extensive (for small concentrations) in 90—88% sulphuric acid. This expectation is fulfilled; but the crude mass-action theory does not quantitatively reproduce the actual extent of conversion in its variation with medium. An empirical equilibrium product, $K$, has been measured for 71·5—100% sulphuric acid media at 25°, with 0·2M initial concentration of guanidine nitrate or nitroguanidine. It has a maximum value in 88% sulphuric acid.

A theoretical connection between $K$ and the water activity has been derived and verified for 71—86% sulphuric acid media, in which nitric acid is only slightly ionised to the nitronium ion.

When dissolved in concentrated sulphuric acid, guanidine nitrate changes into nitroguanidine [cf. Jousselin (who thought the product was nitrosoguanidine), *Compt. rend.*, 1877, **85**, 548; 1879, **88**, 814, 1086; Pellizzari, *Gazzetta*, 1891, **21**, II, 405; and, especially, Thiele, *Annalen*, 1892, **270**, 1]. This transformation is a common method for preparing nitroguanidine (*e.g.*, Ewan and Young, *J. Soc. Chem. Ind.*, 1921, **40**, 109T; Smith, Sabetta, and Steinbach, *Ind. Eng. Chem.*, 1931, **23**, 1124; Davis, *Org. Synth.*, Coll. Vol. I, 1941, pp. 302, 399), the product being precipitated on dilution of the reaction mixture with water. The overall reaction to form free nitroguanidine is given by equation (1):

$$H_2\overset{+}{N}{:}C(NH_2)_2\}NO_3^- \overset{H_2SO_4}{=} HN{:}C(NH_2)NH{\cdot}NO_2 + H_2O \quad . \quad . \quad . \quad (1)$$

That the process is a nitration, and not a dehydration, has been recognised explicitly (*e.g.*, by Davis and Elderfield, *J. Amer. Chem. Soc.*, 1933, **55**, 731).

Barton, Hall, and Wright (*ibid.*, 1951, **73**, 2201) have given reasons for supposing that the nitro-group is attached to the *imino-nitrogen* atom in free nitroguanidine, and not to the amino-nitrogen as represented in equation (1). However, the distinction is immaterial in sulphuric acid solution, because the most probable structures, $[H_2N{:}C(NH_2)NH{\cdot}NO_2]^+$ and $[(H_2N)_2C{:}NH{\cdot}NO_2]^+$, of the ionised forms derived from the two possible formulæ for free nitroguanidine are identical apart from the formal placement of the charge.

Nitroguanidine is a base (though enormously weaker than guanidine; cf. Hall and Sprinkle, *ibid.*, 1932, **54**, 3469) and forms salts, though these are unstable in water (Thiele, *loc. cit.*; Davis, Ashdown, and Couch, *J. Amer. Chem. Soc.*, 1925, **47**, 1063). To precipitate nitroguanidine as free base from sulphuric acid solution, enough water must be added to dilute the sulphuric acid to not more than 20% concentration. If dilution is insufficient, a nitroguanidine sulphate is precipitated. In solution in concentrated sulphuric acid, therefore, nitroguanidine must exist principally as nitroguanidinium ion [equation (5) below]. Guanidine is, of course, a very strong base and will always be present as guanidinium ion (Hall and Sprinkle, *loc. cit.*; Davis and Elderfield, *ibid.*, p. 3470; Angyal and Warburton, *J.*, 1951, 2492).

Although nitroguanidine may be prepared in 90% yield by reaction (1) in sulphuric acid, there are indications that the transformation is reversible in this solvent (as with some other nitramines; cf. Allenby and Wright, *Canad. J. Res.*, 1947, **25**, *B*, 295; Stuart and Wright, *ibid.*, 1948, **26**, *B*, 401; Lamberton, *Quart. Reviews*, 1951, **5**, 75): (1) Davis and Abrams (*Proc. Amer. Acad. Arts Sci.*, 1926, **61**, 437) found that nitroguanidine in

concentrated sulphuric acid acted as a nitrating agent towards aniline, phenol, and aceto-*p*-toluidide. The nitrating action has recently been confirmed (personal communication from Dr. A. H. Lamberton). Davis and Abrams did not ascribe the nitrating action to nitric acid formed reversibly from nitroguanidine; for no nitric acid could be distilled from a solution of nitroguanidine in concentrated sulphuric acid; instead, strong heating gave the decomposition products, nitrous oxide, carbon dioxide, and ammonia (Davis, *J. Amer. Chem. Soc.*, 1922, **44**, 868). However, nitric acid *present in small concentration* is not readily recoverable by distillation from concentrated sulphuric acid (Bennett, Brand, and Williams, *J.*, 1946, 870), owing to the formation of nitronium ion. (2) In sulphuric acid solution, nitroguanidine behaves like nitric acid towards analytical reagents. It reacts quantitatively with mercury and sulphuric acid in the nitrometer (Cope and Barab, *J. Amer. Chem. Soc.*, 1916, **38**, 2552); it also responds quantitatively, like nitric acid, to electrometric titration with ferrous sulphate in sulphuric acid (Cottrell, MacInnes, and Patterson, *Analyst*, 1946, **71**, 207). Some results obtained in the present work, by the method of Treadwell and Vontobel (see Experimental section) are given in Table 1.

TABLE 1. *Titration of solutions of nitroguanidine in concentrated sulphuric acid with ferrous sulphate.*

| | | | |
|---|---|---|---|
| Concn. of nitroguanidine, M | 0·366 | 0·415 | 0·526 |
| Nitrate found, M | 0·377 | 0·414 | 0·524 |

The reversible interconvertibility of guanidine nitrate and nitroguanidine, in sulphuric acid, is proved explicitly by the following experiments, in which a solution, held at constant temperature, was analysed for nitric acid at known time intervals, by a method which made it possible to determine the nitric acid concentration in a sulphuric acid solution which also contained nitroguanidine. Details are given in the Experimental section.

TABLE 2. *The establishment of equilibrium at 25°.*

| Initial concn. of guanidine nitrate = 0·2000M. | | | | | | Initial concn. of nitroguanidine = 0·2000M. | |
|---|---|---|---|---|---|---|---|
| Expt. 78 Medium: 100% $H_2SO_4$. | | Expt. 82 Medium: 87·8% $H_2SO_4$. | | Expt. 27 Medium: 97·2% $H_2SO_4$. | | Expt. 29 Medium: 97·2% $H_2SO_4$. | |
| Time (mins.) | $HNO_3$, M | Time (mins.) | $HNO_3$, M | Time (mins.) | $HNO_3$, M | Time (mins.) | $HNO_3$, M |
| 0 | 0·2000 | 0 | 0·2000 | 0 | 0·2000 | 0 | 0 |
| 8·7 | 0·1674 | 9·2 | 0·0126 | 2·4 | 0·1124 | 1·8 | 0·1129 |
| 12·2 | 0·1672 | 13·0 | 0·0122 | 5·3 | 0·1133 | 5·7 | 0·1133 |
| 17·0 | 0·1679 | 16·5 | 0·0124 | 9·0 | 0·1130 | 9·2 | 0·1134 |
| 20·8 | 0·1671 | 21·4 | 0·0127 | 12·7 | 0·1122 | 13·3 | 0·1140 |
| 27·6 | 0·1666 | 27·2 | 0·0124 | 19·1 | 0·1121 | 19·7 | 0·1140 |
| 34·1 | 0·1650 | 36·3 | 0·0125 | 32·1 | 0·1121 | 36·0 | 0·1138 |
| 67·4 | 0·1672 | 52·3 | 0·0125 | 55·8 | 0·1105 | 59·3 | 0·1119 |

*Reversibility of the Reaction.*—When either guanidine nitrate, or an equivalent concentration of nitroguanidine, is dissolved in the sulphuric acid medium, the nitric acid concentration moves rapidly to a constant value which is the same with either starting material. The composition of the equilibrium mixture depends on the medium composition. The experimental proof is given in Fig. 1; and other sample experiments are shown in Table 2.

*Rates of Reaction.*—Expts. 15/16 and 23/24 (see Fig. 1) show that the attainment of equilibrium is very rapid in 85·6% sulphuric acid with 0·027M-reagents. With higher concentrations of guanidine nitrate (*e.g.*, 0·2M), the material takes appreciable time (up to 10 minutes) to dissolve in media weaker than 90% sulphuric acid, though the first measurement made on the homogeneous solution corresponds to the equilibrium composition. It may be assumed, therefore, that the actual chemical reaction is very rapid in media containing more than 85% sulphuric acid. With 0·018M-reagents in 80·7% sulphuric acid (Expts. 18/19), however, the approach to equilibrium takes a measurable time, being incomplete after 1 hour.

*Notation.*—In the following, the symbols G and P stand for the free base molecules of

guanidine and nitroguanidine, respectively. The symbol $[HNO_3]$ represents the stoicheiometric concentration of nitric acid, as determined by analysis; so that $[HNO_3] = [NO_2^+] + [NO_2{\cdot}OH]$, if neither $H_2NO_3^+$ nor $NO_3^-$ is formed. All concentrations are recorded in moles per litre.

*Mass-action Considerations.*—It is convenient to represent the results of the measurements in terms of an equilibrium product, $K$, defined by the equation:

$$K = [PH^+]_e/[GH^+]_e[HNO_3]_e \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

$K$ is thus defined by measurable concentrations. $[GH^+]$ and $[PH^+]$ are, respectively, the concentrations of guanidinium and nitroguanidinium ions. The suffix $e$ refers to equilibrium concentrations.

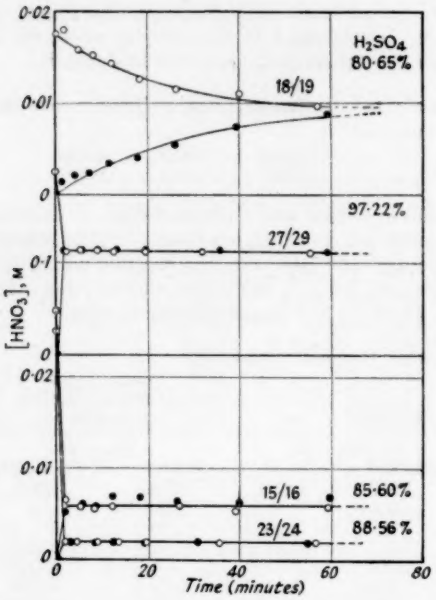



FIG. 1. *The guanidine nitrate–nitroguanidine equilibrium.*

○, *With initial guanidine nitrate.*
●, *With initial nitroguanidine.*

The overall conversion into neutral nitroguanidine is represented by equation (1). In a medium of concentrated sulphuric acid, however, the following equilibria are set up:

$$NO_3^- + H_2SO_4 \rightleftharpoons NO_2{\cdot}OH + HSO_4^- \quad . \quad . \quad . \quad . \quad (3)$$

$$NO_2{\cdot}OH + 2H_2SO_4 \rightleftharpoons NO_2^+ + OH_3^+ + 2HSO_4^- \quad . \quad . \quad . \quad (4)$$

$$HN{:}C(NH_2){\cdot}NH{\cdot}NO_2 + H_2SO_4 \rightleftharpoons \overset{+}{H_2}N{:}C(NH_2){\cdot}NH{\cdot}NO_2 + HSO_4^- \quad . \quad (5)$$

$$H_2O + H_2SO_4 \rightleftharpoons OH_3^+ + HSO_4^- \quad . \quad . \quad . \quad . \quad . \quad (6)$$

Of these, (3) and (5) probably lie completely, and (6) probably lies substantially, to the right in most of the media considered. The position of (4) depends on the composition of the system. Further equations will be needed if guanidine takes up more than one proton in very concentrated sulphuric acid (see below), and if nitric acid forms any appreciable concentration of nitrate ion in the most aqueous media.

By combining these equations with (1), the nitroguanidine equilibrium may be written:

$$GH^+ + NO_2^+ + HSO_4^- \rightleftharpoons PH^+ + H_2SO_4 \quad . \quad . \quad . \quad (7)$$

Equation (7) is based on the assumption that the nitronium ion is the sole nitrating agent. This will be true in media containing 95% of sulphuric acid, or more, in which

equilibrium (4) lies completely to the right (for relatively small concentrations of nitric acid). Whether $NO_2^+$ is the sole nitrating agent in more aqueous media, or whether the $H_2NO_3^+$ ion (if present) or the $NO_2{\cdot}OH$ molecule can also nitrate the guanidinium ion, are matters for investigation. The nitronium ion is formed in sufficient concentration in 75—82% sulphuric acid to be effective in aromatic nitration (Lowen, Murray, and Williams, *J.*, 1950, 3318). Velocity measurements with guanidine nitrate, in 71·5—83% sulphuric acid, furnish evidence that the nitronium ion nitrates guanidine in the same media (unpublished experiments).

From equation (7) an equilibrium constant, $K_0^\circ$, may be defined by equation (8):

$$K_0^\circ = \frac{[PH^+]_e[H_2SO_4]_e}{[GH^+]_e[NO_2^+]_e[HSO_4^-]_e} \cdot \frac{f_{PH^+}}{f_{GH^+}} \cdot \frac{f_{H_2SO_4}}{f_{NO_2^+} f_{HSO_4^-}} \quad . \quad . \quad . \quad . \quad (8)$$

If the term in activity coefficients is neglected for the time being, then from (2) and (8)

$$K = K_0 r Q \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

in which $K_0$ is the " concentration " equilibrium constant for (7), and

$$r = [HSO_4^-]/[H_2SO_4]; \; Q = [NO_2^+]/[HNO_3] \quad . \quad . \quad . \quad . \quad (10)$$

Equation (9) can be made to show how the empirical equilibrium product, $K$, may be expected to vary with medium composition. For *small* reagent concentrations, $r \sim r_m$, in which $r_m$ is the ratio $[HSO_4^-]/[H_2SO_4]$ for the sulphuric acid–water medium. In equilibrium (6), $r_m$ is not known with certainty over any extensive medium range; but Brand has shown (*J.*, 1950, 997) that the equation ($H_0$ being Hammett's acidity function)

$$\log r_m = H_0 + 8{\cdot}35 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

is valid for 87—99·8% sulphuric acid when $r_m$ is calculated on the supposition that water reacts quantitatively in (6), to form oxonium and bisulphate ions. If equation (11) can be supposed to define $r_m$ in still more aqueous media [in which (6) certainly does not lie quantitatively to the right], then the calculated $r_m$ values may be combined with estimates of $Q$ derived from the combination of carbinol indicator and aromatic nitration measurements (Bonner and Williams, *Chem. and Ind.*, 1951, 820), to give the products $r_mQ$ for insertion in (9). Relative values of $r_mQ$ are shown in Table 3.

TABLE 3. *Estimate of medium effect on K.*

| Medium, $H_2SO_4$, % | 99 | 95 | 90 | 89 | 88 | 87·5 | 87 | 85 | 82 |
|---|---|---|---|---|---|---|---|---|---|
| $r_mQ$ (rel.) ..... | 0·076 | 0·52 | 1·2 | 1·2 | 1·1 | 1·0 | 0·78 | 0·36 | 0·095 |
| $K$ (obs. rel.) ..... | 0·0017 | 0·022 | 0·54 | 0·83 | 0·98 | 1·0 | 0·92 | 0·53 | 0·23 |

*Influence of Medium Composition: Experimental Results.*—Results for the equilibrium product, $K$, in different media, are recorded in Tables 4 and 5. All these results were determined from the equilibrium compositions derived from 0·2M-starting materials. With this reagent concentration it is not very accurate to set $r \sim r_m$ in equation (9). Nevertheless, relative values of $K$ are put in Table 3 for comparison with the calculated $r_mQ$. Quantitatively, equation (9) does not reproduce the decrease of $K$ from the maximum with any accuracy, particularly in media more acid than the optimum, although it predicts a maximum value for $K$ in about the right medium. $K_0$ in (9) is not independent of medium composition.

The results in Table 5 are for media in which the approach to equilibrium is slow enough to be followed from either side. Starting materials for nitration were mixed solutions of guanidine carbonate and nitric acid in the sulphuric acid medium. The values of $K$ in Table 5, like those in Table 4, are calculated from equilibrium end-point measurements.

*Equilibrium Products from Initial-rate Measurements.*—In the more aqueous media (Table 5) it is also possible to calculate $K$ as the quotient, $k_2/k_1$, of velocity coefficients for initial rates of nitration of the guanidinium ion and of denitration of nitroguanidine. Equilibrium products so derived are shown as blackened circles in Fig. 2. They are in satisfactory agreement with the equilibrium products determined from end-point measurements.

TABLE 4. *Influence of medium composition (rapid reactions) at 25°.*

Initial concentration of guanidine nitrate, 0·2000M.

| Expt. no. | Medium: $H_2SO_4$, % | At equilibrium: $HNO_3$, M | Nitroguanidine, M | Conversion into nitroguanidine, % | $K$ | $K$ (mean) |
|---|---|---|---|---|---|---|
| 76 | 100 | 0·1668 | 0·0332 | 16·6 | 1·19 | |
| 77 | — | 0·1682 | 0·0318 | 15·9 | 1·12 | 1·17 |
| 78 | — | 0·1669 | 0·0331 | 16·6 | 1·19 | |
| 42 | 98·95 | 0·1536 | 0·0464 | 23·2 | 1·97 | |
| 43 | — | 0·1551 | 0·0449 | 22·5 | 1·87 | 1·92 |
| 134 | 98·87 | 0·1464 | 0·0536 | 26·8 | — | 2·50 |
| 110 | 98·64 | 0·1527 | 0·0473 | 23·7 | — | 2·03 |
| 154 | 98·40 | 0·1436 | 0·0564 | 28·2 | — | 2·74 |
| 27 | 97·22 | 0·1122 | 0·0879 | 43·9 | 6·98 | |
| 28 | — | 0·1116 | 0·0886 | 44·2 | 7·12 | 7·08 |
| 32 | — | 0·1114 | 0·0886 | 44·3 | 7·15 | |
| 117 | 97·11 | 0·1195 | 0·0805 | 40·2 | — | 5·64 |
| 49 | 95·74 | 0·0888 | 0·1112 | 55·6 | 14·1 | |
| 51 | — | 0·0883 | 0·1117 | 55·9 | 14·3 | 14·2 |
| 58 | 93·95 | 0·0534 | 0·1466 | 73·3 | 51·4 | |
| 59 | — | 0·0536 | 0·1464 | 73·2 | 51·0 | 51·2 |
| 149 | 93·85 | 0·0506 | 0·1494 | 74·7 | — | 58·3 |
| 139 | 91·60 | 0·0261 | 0·1739 | 87·0 | — | 255 |
| 64 | 90·95 | 0·0216 | 0·1784 | 89·2 | 383 | |
| 65 | — | 0·0208 | 0·1792 | 89·6 | 414 | 399 |
| 70 | 88·61 | 0·0135 | 0·1865 | 93·3 | — | 1024 |
| 79 | 87·84 | 0·0131 | 0·1869 | 93·5 | 1088 | |
| 80 | — | 0·0134 | 0·1866 | 93·3 | 1044 | |
| 81 | — | 0·0127 | 0·1873 | 93·7 | 1166 | 1121 |
| 82 | — | 0·0125 | 0·1875 | 93·8 | 1205 | |
| 83 | — | 0·0130 | 0·1870 | 93·5 | 1111 | |
| 71 | 87·16 | 0·0130 | 0·1870 | 93·5 | — | 1113 |
| 73 | 86·70 | 0·0142 | 0·1858 | 92·9 | — | 916 |
| 102 | 85·03 | 0·0171 | 0·1829 | 91·5 | — | 628 |
| 74 | 84·90 | 0·0180 | 0·1820 | 91·0 | 560 | |
| 75 | — | 0·0186 | 0·1814 | 90·1 | 526 | 543 |
| 144 | 83·79 | 0·0202 | 0·1798 | 89·9 | — | 441 |
| 94 | 83·09 | 0·0215 | 0·1785 | 89·3 | 385 | |
| 95 | — | 0·0216 | 0·1784 | 89·2 | 384 | 385 |
| 100 | 81·84 | 0·0270 | 0·1730 | 86·5 | 237 | |
| 101 | — | 0·0273 | 0·1727 | 86·4 | 233 | 235 |

TABLE 5. *Influence of medium composition (slow reactions) at 25°.*

| Expt. no. | Medium, $H_2SO_4$, % | Concn. of starting materials: $GH^+$, M | $HNO_3$, M | $PH^+$, M | At equilibrium: $HNO_3$, M | % of theor. as $PH^+$ | $K$ |
|---|---|---|---|---|---|---|---|
| 235/7/8 | 82·94 | — | — | 0·2084 | 0·0240 | 86·8 | 294 |
| 160/1 | 81·14 | 0·1907 | 0·1930 | — | 0·0288 | 86·0 | 212 |
| 221/2 | 79·24 | 0·2070 | 0·2093 | — | 0·0413 | 80·1 | 106 |
| 228/9 | 79·24 | — | — | 0·2084 | 0·0370 | 82·9 | 122 |
| 207/13/14 | 75·47 | — | — | 0·2024 | 0·0570 | 72·0 | 44·6 |

*Influence of Medium: Quantitative Treatment.*—From equations (2) and (8),

$$\log K = -pK_0^\circ + \log \frac{[NO_2^+]}{[NO_2^+] + [NO_2{\cdot}OH]} + \log \frac{[HSO_4^-]}{[H_2SO_4]} - \log \left\{ \frac{f_{PH^+}}{f_{GH^+}} \cdot \frac{1}{f_{NO_2^+}} \cdot \frac{f_{H_2SO_4}}{f_{HSO_4^-}} \right\} \quad (12)$$

in which all concentrations refer to the equilibrium state.

If (4) is split formally into (6) and (13)

$$NO_2{\cdot}OH + H_2SO_4 \rightleftharpoons NO_2^+ + HSO_4^- + H_2O \quad . \quad . \quad . \quad . \quad (13)$$

then for (13) (which may have physical significance in the less acid media),

$$\log K_{13}^\circ = \log \frac{[NO_2^+]}{[NO_2OH]} + \log \frac{[HSO_4^-]}{[H_2SO_4]} + \log a_{H_2O} + \log \left\{ \frac{f_{NO_2^+}}{f_{NO_2{\cdot}OH}} \cdot \frac{f_{HSO_4^-}}{f_{H_2SO_4}} \right\} \quad . \quad (14)$$

Media on the aqueous side of the optimum may be treated by putting $[NO_2{\cdot}OH] \gg [NO_2^+]$ in (12). Then, from (12) and (14),

$$\log K = -\mathrm{p}K_0^\circ - \mathrm{p}K_{13}^\circ - \log a_{H_2O} - \log \left\{ \frac{f_{PH^+}}{f_{GH^+}} \cdot \frac{1}{f_{NO_2{\cdot}OH}} \right\} \quad . \quad . \quad (15)$$

The variation of the $f$-term in (15) with medium may be expected to depend mainly on the variation of $f_{NO_2{\cdot}OH}$; and this may be relatively slight in the range 85—70% sulphuric

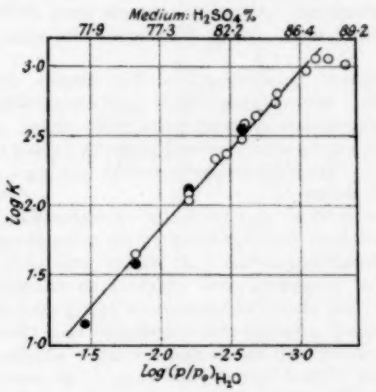



FIG. 2. *Equilibrium product and water activity in sulphuric acid media.*

acid, where the system is always loaded with electrolyte. If $f_{NO_2{\cdot}OH}$ does not vary greatly, then log $K$ should be connected with log $a_{H_2O}$ by a straight line of slope $-1$. Essentially the same result may be obtained from equation (12) and an equation of type (11), together with the equation, $J_0 = -\mathrm{p}K_{NO_2{\cdot}OH} - \log\{[NO_2^+]/[NO_2{\cdot}OH]\}$, defining an acidity function $J_0$ for carbinol ionisation (Lowen, Murray, and Williams, *loc. cit.*), and the alternative (and equivalent) definition, $J_0 = H_0 + \log a_{H_2O}$, of Gold and Hawes (*J.*, 1951, 2102).

In Fig. 2, log $K$ is plotted against log $(p/p_0)$, the vapour-pressure ratio for water over sulphuric acid–water mixtures, as tabulated by Gold and Hawes (*loc. cit.*).* That the points can be connected by a straight line is no matter for surprise; but the gradient of $-1{\cdot}1$ is significant and is consistent with equation (15), implying that equation (12) is a plausible description of the variation of $K$ with medium composition when nitric acid is only slightly ionised to the nitronium ion. The relation in Fig. 2 appears to hold up to a medium strength of 86—87% sulphuric acid, in which the ratio $[NO_2^+]:[HNO_3]$ is probably 0·08—0·16 (Bonner and Williams, *loc. cit.*).

For large ionisations of nitric acid, equation (15) is naturally invalid; and Table 3 has already shown that a simple mass-action theory, based on equations (9—11), is incapable of accounting for the large fall in $K$ in the medium range 88—100% sulphuric acid. According to Hantzsch (*Ber.*, 1930, **63**, 1782), guanidine acts as a triacid base in nearly anhydrous sulphuric acid. The formation of ions $H_2N{:}C(NH_2){\cdot}NH_3^{2+}$ and $H_2N{:}C(NH_3)_2^{3+}$, in the most concentrated sulphuric acid media, would diminish $K$. Even if correct, this is not likely to be the only factor which invalidates the simple mass-action theory in the most acid media.

* In Fig. 2, no allowance can be made for the influence of the reagents (total concn. 0·2M) on log $a_{H_2O}$. The *change* in their influence, over the medium range in question, is possibly not great.

## Experimental

*Materials.*—Pure guanidine nitrate and nitroguanidine were given to us through the kind assistance of Dr. T. M. Walters.

The guanidine nitrate was recrystallised twice from water or methyl or ethyl alcohol and dried at 80—110° for 20 hours before use. It had m. p. 215° (uncorr.) (Found, for two samples: C, 10·35, 10·4; H, 4·9, 5·0; N, 45·2, 45·1%. Calc. for $CH_6O_3N_4$: C, 9·8; H, 4·95; N, 45·9%). Its nitrate content was tested by the analytical method described below (Calc. for an aqueous solution: $[NO_3^-] = 0{\cdot}0870$M. Found: 0·0875, 0·0875, 0·0875, 0·0877, 0·0879, 0·0873, 0·0875M; mean, 0·0876M).

The nitroguanidine [m. p. 224—226° (decomp.)] was dried over phosphoric oxide for 17 hours at 65°/10 mm. Recrystallisation from boiling water or other solvents was deleterious. Freedom of the material from inorganic impurity is shown by its quantitative titration with ferrous sulphate in concentrated sulphuric acid (see Table 1).

Sulphuric acid media were standardised against N-sodium hydroxide, referred, through N-hydrochloric acid, to potassium iodate as ultimate standard. Each medium was analysed by six titrations. A typical example gave 82·77, 82·69, 82·85, 82·89, 82·76, 82·93, mean 82·81% of sulphuric acid. 100% Sulphuric acid was prepared by adjustment to maximum freezing point.

*Analytical Solutions.*—For the nitrate determinations, a saturated stock solution of "AnalaR" ferrous ammonium sulphate in N-sulphuric acid was diluted with N-sulphuric acid to the appropriate concentration (0·05—0·5N), and standardised against potassium dichromate. Identical values were obtained with the "dead stop" end-point (see below) and with "ferroin" indicator. The dichromate solution was used as a direct standard and cross-checked against standard iodate.

*Determination of Equilibrium Composition.*—A known weight of guanidine nitrate (or nitroguanidine) was introduced from a weighing bottle (fitted with B.24 cone, and socket cap) by inverting this in the B.24 socket neck of a flask of 100-c.c. capacity, containing a known weight of sulphuric acid medium at thermostat temperature. With about 0·2M-reagent, solution took about 15 seconds in 100% and about 9 minutes in 81·9% sulphuric acid. The homogeneous solution was transferred to a three-necked reaction vessel, carrying a stirrer and a thermometer well, in the thermostat. Samples (5 ml.) were extracted at intervals, through the third neck (closed by a ground cap), to be analysed for nitric acid, by means of a pipette (pre-heated to 25°) fitted with a tap above the mark. Since it was the final equilibrium composition which had to be measured, and not the speed of attainment of equilibrium, it was essential only to ensure that the *final* solution was accurately at 25° (more exactly, 24·91°).

Nitroguanidine could not be dissolved, in the above manner, in sulphuric acid stronger than 98%, because the heat of solution caused decomposition.

Volumetric glassware was calibrated. Pipettes for sampling were calibrated to deliver sulphuric acid solutions at the appropriate temperature.

*Analytical Methods.*—The very convenient electrometric titration with ferrous sulphate in concentrated sulphuric acid (Treadwell and Vontobel, *Helv. Chim. Acta*, 1937, **20**, 573) cannot be used for nitric acid in presence of nitroguanidine (Table 1). A modification of the method of Kolthoff, Sandell, and Moskovitz (*J. Amer. Chem. Soc.*, 1933, **55**, 1454; cf. Williams and Lowen, *J.*, 1950, 3312) can be employed instead.

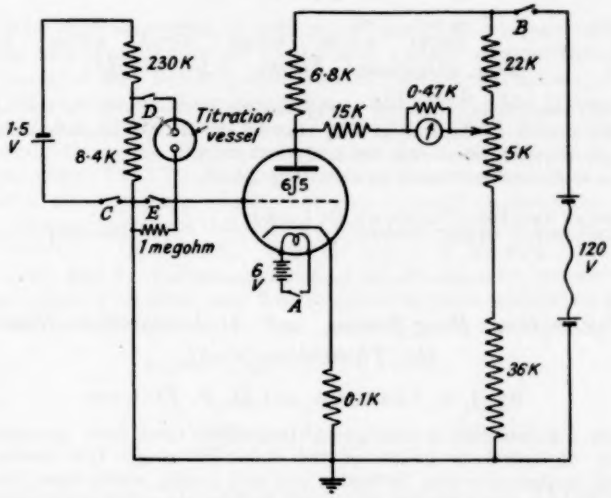
In this method, nitrate is reduced to nitric oxide, in the absence of air, by boiling it with excess of standard ferrous sulphate, in hydrochloric acid medium, with ammonium molybdate as catalyst. The reaction is completed by expelling nitric oxide; and the residual ferrous sulphate is titrated with potassium dichromate. For the nitroguanidine reaction, this method has the advantage that the sample may be drowned in water, to freeze the equilibrium composition. However, nitroguanidine is partly decomposed, during this process of analysis, forming nitrate, and causing a 5% error.

It is therefore necessary to destroy the nitroguanidine present, before analysing for nitrate. This can be done by the action of hot alkali, which decomposes nitroguanidine into nitrous oxide, ammonia, and carbon dioxide (Franchimont, *Rec. Trav. chim.*, 1891, **10**, 233; Thiele, *loc. cit.*). Excess of alkali advantageously stabilises nitric acid as metallic nitrate, while the nitroguanidine is being destroyed. The following standardised procedure has given successful results.

*Procedure.* The 5-ml. sample of sulphuric acid medium, containing nitric acid and nitroguanidine, is run into 55 ml. of water, and the walls of the vessel are washed with a further

15 ml. of water. After 2 minutes, 30 ml. of 50% (w/v) sodium hydroxide solution are added. (The solution may be safely preserved for analysis in this condition.) The solution is boiled (total heating time, 12 minutes), to destroy nitroguanidine. It is then cooled in ice-water, in an atmosphere of carbon dioxide. After 2·5 minutes' cooling, the sample is acidified with 70 ml. of 36% hydrochloric acid. An inert atmosphere in the flask is conserved by carbon dioxide evolved from sodium carbonate formed during the previous operation. To the acid solution, 15 ml. of standard ferrous ammonium sulphate solution (0·05—0·5N) are added from an automatic burette. The flask is fitted with a rubber cork carrying a tap-funnel and an exit tube. When the (yellow) solution starts to boil (3—4 minutes), a 1% solution of ammonium molybdate (about 6 ml.) is run in. The colour of the solution changes to green and, after further boiling, to orange, which signifies that the reaction between ferrous ion and nitrate is complete. Boiling is continued until a total time of 24 minutes has passed. The solution is cooled in ice-water (inert atmosphere) for 4 minutes to about 10°; and then transferred to a beaker. The volume is made up to 140 ml. with water (to dissolve salts precipitated during the previous concentration), and the excess of ferrous ion in the solution is titrated with standard potassium

FIG. 3.




dichromate delivered from a micro-burette. The end-point is detected by the electrometric "dead-stop" method (Foulk and Bowden, *J. Amer. Chem. Soc.*, 1926, **48**, 2045), which has been made more sensitive by a D.C. amplifier. A potential difference of the order 15 mv. is applied across two platinum-wire electrodes in the acid ferrous salt solution being titrated. During titration the potential difference across the electrodes is applied as a potential to the grid of a triode valve. Changes in the anode potential are recorded on a galvanometer, which registers a "kick" of increasing magnitude (up to 80 scale-divisions) as the end-point is approached. As soon as the end-point is passed, the galvanometer needle swings in the opposite direction. The circuit is shown in Fig. 3.

During titration the solution is mechanically stirred and a carbon dioxide atmosphere is maintained above the liquid.

Solutions of widely varying concentrations may be analysed by this method, but at low concentrations (0·02M) a "blank correction" (equivalent to 0·0015M-nitrate) becomes an appreciable fraction of the ferrous ion consumed. The correction was applied to all titrations to allow for "blank" loss of ferrous ion which occurred during the (standardised) process of analysis and could not be eliminated. The "blank" correction amounts to some 10% of the equilibrium nitrate concentration when $K$ is near its maximum (Table 4). For other conditions it is much less significant.

When a series of samples is to be analysed, the operations may be conveniently staggered by a routine process. Eight samples can be analysed in 2 hours.

*Tests of Method.*—(a) *On solutions in sulphuric acid.* Solutions containing potassium nitrate, with or without nitroguanidine, in concentrated sulphuric acid, gave the following results:

| Nitroguanidine, M | 0 | 0 | 0 | 0·5 | 0 | 0·5 | 0 | 0·5 |
|---|---|---|---|---|---|---|---|---|
| Nitrate found, M | 0·1349 | 0·1328 | 0·1321 * | 0·1344 | 0·1327 | 0·1372 * | 0·1341 | 0·1341 |

Mean, nitrate found: 0·1340M. Calc., 0·1339M.

These were early results, obtained before certain refinements of method had been introduced. The mean error of the eight estimations is 0·87%, or, by disregard of the values marked with an asterisk, 0·52%.

(b) *On aqueous potassium nitrate solutions.* These contained no nitroguanidine, and the alkali treatment was omitted. Nitrate found: 0·0995, 0·0993, 0·0977, 0·0999, 0·0991; mean 0·0991M. Calc.: 0·0990M.

(c) *On aqueous solutions of guanidine nitrate.* (i) Figures obtained without alkali treatment have already been given in the discussion of the purity of the guanidine nitrate.

(ii) The following results were obtained by subjecting aqueous solutions of guanidine nitrate (with or without nitroguanidine) to the whole analytical procedure, including alkali treatment:

| Nitroguanidine, M | 0 | 0·4 | 0 | 0·4 | 0 | 0 | 0·4 |
|---|---|---|---|---|---|---|---|
| Nitrate found, M | 0·0784 | 0·0786 | 0·0788 | 0·0795 | 0·0791 | 0·0789 | 0·0781 |

Mean, nitrate found: 0·0788M. Calc.: 0·0793M.

These final results confirm (*a*) that the nitroguanidine is effectively destroyed and does not interfere with the nitrate estimation, and (*b*) that no nitrate is artificially created by decomposition of the guanidinium ion during the analytical process.

The accuracy of the method seems to be within ±0·8%.

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[*Received, March 28th*, 1952.]

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## 593. *Cyclisation, Ring-fission, and Acyl-migration Reactions in the Thiazoline Field.*

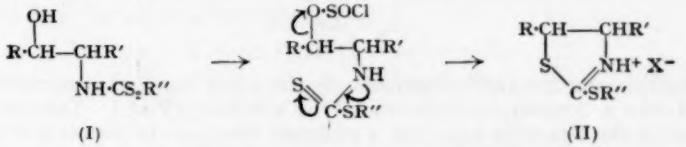
By J. C. CRAWHALL and D. F. ELLIOTT.

Some *S*-substituted 2-mercapto-$\Delta^2$-thiazolines have been prepared for a study of hydrolytic fission of the thiazoline ring. The mechanisms involved in thiazoline ring formation and ring fission, which have been discussed, have a direct bearing on recent theories regarding acyl migrations and intramolecular displacements in molecules containing contiguous amino- and hydroxy-groups. Somewhat unexpected was the discovery of the dual mechanism involved in the cyclisation of certain *N*-thioacylamino-alcohols with thionyl chloride; this demonstrates the need for caution in the stereochemical interpretation of results obtained with this reagent in the field of acylated amino-alcohols. During this work some dithiolcarbonic acid derivatives and 2-ketothiazolidines have been prepared.

LINDERSTRÖM-LANG and JACOBSEN (*Compt. rend. Lab. Carlsberg, Sér. chim.*, 1940, **23**, 289) studied the fission of the ring in 2-methyl-$\Delta^2$-thiazoline under hydrolytic conditions and found that the main product was 2-acetamidoethanethiol, resulting from cleavage of the ring between the sulphur atom and the 2-carbon atom, but the presence of a certain amount of 2-acetylthioethylamine in the product was inferred from analytical data. Recently, an example of exclusive cleavage of the $\Delta^2$-thiazoline ring between $C_{(2)}$ and the nitrogen atom was recorded (Crawhall and Elliott, *J.*, 1951, 2071). It was of interest, therefore, to prepare a number of benzylthio- and methylthio-$\Delta^2$-thiazolines and study their reactions. During this work some facts emerged which have a direct bearing on the various mechanisms involved in intramolecular displacements and acyl migrations in amino-alcohols. Some observations have also been made on the structure of compounds prepared by

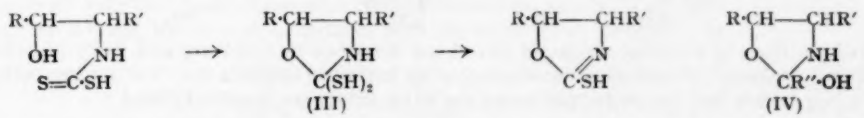
Niederl and Hart (*J. Amer. Chem. Soc.*, 1942, **64**, 2487), and considered to be 2-arylthio-$\Delta^2$-thiazolines.

$\Delta^2$-Thiazolines (cf. II) were prepared from ethanolamine, 2-aminopropan-1-ol, 1-aminopropan-2-ol, and 3-aminobutan-2-ol by conversion into esters (I) of dithiocarbamic acid by carbon disulphide and benzyl chloride or methyl iodide in the presence of triethylamine and pyridine, followed by cyclisation with thionyl chloride (cf. Crawhall and Elliott, *loc. cit.*). This reagent generally gave better results than either phosphorus tribromide or alcoholic hydrogen chloride, although competing reactions were observed in two instances (see below).



(I) (II)

It is noteworthy that, when treated with carbon disulphide and alcoholic alkali, aminoalcohols carrying substituents attached to the same carbon atom as the hydroxyl group are generally converted into 2-mercapto-oxazolines (Bruson and Eastes, *J. Amer. Chem. Soc.*, 1937, **59**, 2011; Hopkins, *Canadian J. Res.*, 1942, **20**, *B*, 268; Maquenne and Roux, *Compt. rend.*, 1902, **134**, 1589; Roux, *Ann. Chim.*, 1904, **1**, 72). Ethanolamine, however, gives 2-mercapto-$\Delta^2$-thiazoline (Knorr and Roessler, *Ber.*, 1903, **36**, 1278). Ettlinger (*J. Amer. Chem. Soc.*, 1950, **72**, 4792) has suggested that the thiazoline is produced by intermediate formation of a xanthate which undergoes internal displacement by the dithiocarbamate ion; the mechanism of this reaction would be analogous to that already postulated for the conversion (I) $\longrightarrow$ (II). Xanthic esters of secondary alcohols (Laakso, *Suomen Kem.*, 1940, **133**, 8; Trieber, *Monatsh.*, 1951, **82**, 53) may be more slowly formed than those from primary alcohols, and 2-mercapto-oxazolines could then be formed by a competing reaction as follows:

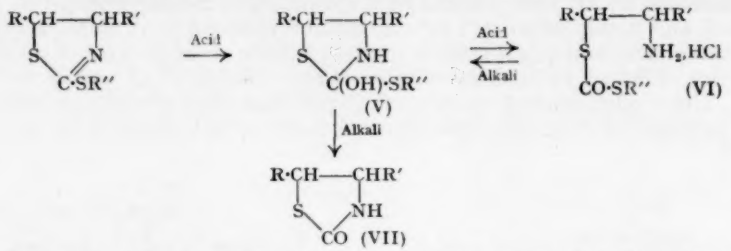


(III) (IV)

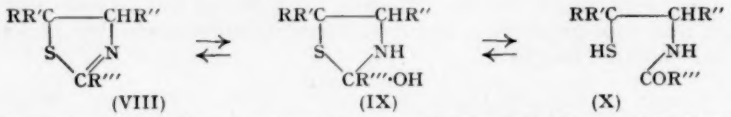
The hypothetical substance (III) closely resembles a compound (IV) first suggested by Bergmann, Brand, and Weinmann (*Z. physiol. Chem.*, 1923, **131**, 1) as the intermediate in the O $\longrightarrow$ N migration of acyl groups in acylated 1 : 2-amino-alcohols. This structure has recently been considered by a number of workers (Phillips and Baltzly, *J. Amer. Chem. Soc.*, 1947, **69**, 200; Fry, *J. Org. Chem.*, 1950, **15**, 802; Winstein and Boschan, *J. Amer. Chem. Soc.*, 1950, **72**, 4669) and, as Phillips and Baltzly (*loc. cit.*) have pointed out, is probably the intermediate in N $\longrightarrow$ O acyl migration under certain conditions. As a result of the work now described, additional evidence has been obtained that compounds of this structure play an important rôle in acyl migration and related reactions.

The benzylthio- and 2-methylthio-$\Delta^2$-thiazolines, prepared as described above, were all cleaved by hot aqueous acid between the $C_{(2)}$ and the nitrogen atom, yielding derivatives (VI) of dithiolcarbonic acid which were very resistant to further hydrolysis. Purification of the compounds derived from 3-aminobutan-2-ol (cf. VI; R = R′ = Me, R″ = $CH_2Ph$) and 1-aminopropan-2-ol (cf. VI; R = Me, R′ = H, R″ = $CH_2Ph$) was complicated by the presence of other substances described below. The basic properties of (VI; R = R′ = H, R″ = $CH_2Ph$), which can be regarded as representative of this class of compound, were demonstrated by the formation of an acetyl and a formyl derivative. The free bases derived from the hydrochlorides (VI) could not be prepared because in solution above pH 7 immediate liberation of a thiol took place and 2-ketothiazolidine (2-hydroxythiazoline) derivatives (VII) were formed. This reaction could be carried out in the pre-

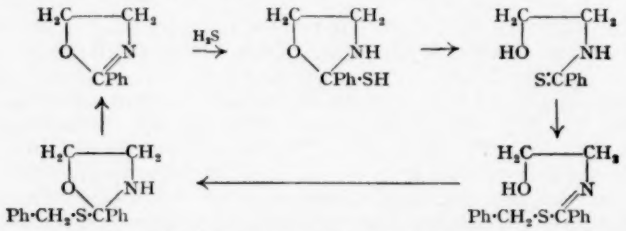
sence of aqueous sodium hydrogen carbonate solution or in a solution of triethylamine in ethanol. These transformations can be explained by the following reaction scheme:




In the 2-alkyl- and 2-aryl-$\Delta^2$-thiazolines on the other hand, the intermediate (IX) is converted into a 2-mercapto-amide under the influence of acid. This shows that the substituent in the 2-position can exert a profound effect on the direction of ring opening. An additional example of ring fission between the sulphur atom and $C_{(2)}$ has been found in this laboratory: 2-phenylthiazoline-4-carboxylic acid (VIII; R = R′ = H, R″ = $CO_2H$, R‴ = Ph) (Crawhall and Elliott, *loc. cit.*) was rapidly hydrolysed by boiling water, yielding *N*-benzoylcysteine in almost quantitative yield. (This is a convenient method of preparing the rather inaccessible cysteine derivative.) The reversibility of the reaction just described has been demonstrated in derivatives of penicillamine (X; R = R′ = Me, R″ = $CO_2H$, R‴ = Me or Ph), which are converted by hydrogen chloride in anhydrous solvents into the corresponding thiazolines (Merck and Co. Inc., C.P.S. 237, July 24, 1944); (X; R = R′ = Me, R″ = $CO_2H$, R‴ = Ph) shows the remarkable property of being cyclised by boiling aqueous hydrochloric acid. These transformations can be interpreted as follows:



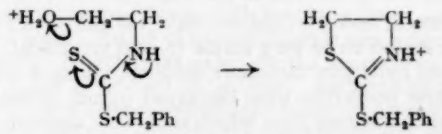

Two reactions of a similar character have been described by Goldberg and Kelly (*J.*, 1948, 1919): 2-phenyl-$\Delta^2$-oxazoline was converted by hydrogen sulphide into *N*-2′-hydroxyethylthiobenzamide and was re-formed when the latter substance was benzylated:



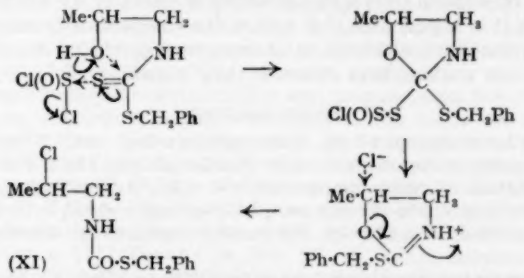

The cyclisation of the thioimidate is analogous to the formation of oxazolines or thiazolines by reaction of 1:2-hydroxy- or 1:2-mercapto-1-amino-compounds with imidic esters (Barber, Gregory, Slack, Stickings, and Woolman, C.P.S. 66, May 24th, 1944; Elliott, *J.*, 1949, 589; Crawhall and Elliott, *loc. cit.*). It will be seen that thiazoline ring formation can take place by two different mechanisms, exemplified by the reactions (I) ⟶ (II) and (X) ⟶ (VIII) or (VI) ⟶ (VII). The essential step in all the above examples, except the reaction (I) ⟶ (II), is the addition of a nucleophilic group to the carbon atom of a carbonyl or thiocarbonyl group. Reactions closely related to these have been described by Davis and Levy (*J.*, 1951, 2419) and Waley and Watson (*Proc. Roy. Soc.*, 1949, *A*, **199**, 499). The first step in the fission of the $\Delta^2$-thiazoline ring is the addition of a hydroxyl group to $C_{(2)}$; the nature of the 2-substituent determines the direction of ring opening. In

the $\Delta^2$-oxazoline series ring fission can occur by a reaction which can be considered as a reversal of the reaction (I) ⟶ (II), namely, nucleophilic attack on $C_{(5)}$ (Fry, *J. Org. Chem.*, 1949, **15**, 438, 802; Winstein and Boschan, *loc. cit.*), but there appear to be no examples of thiazoline ring fission by this mechanism.

Two rather unexpected reactions have been observed during this work. 2-*N*-Dithiocarbobenzyloxyaminoethanol (I; R = R′ = H, R″ = $CH_2Ph$) on treatment with cold ethanolic hydrogen chloride gave 2-benzylthio-$\Delta^2$-thiazoline (II; R = R′ = H, R″ = $CH_2Ph$) in excellent yield; from the same reagents in the hot, 2-aminoethyl benzyl dithiolcarbonate hydrochloride (VI; R = R′ = H, R″ = $CH_2Ph$) was obtained, presumably as a result of cleavage of the thiazoline ring. Cyclisation must have occurred after activation of the hydroxyl group as a hydroxonium ion :



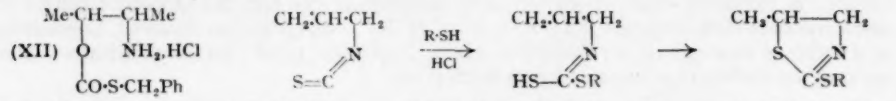
It might have been expected that an oxazoline would have been formed by the alternative mechanism [*e.g.*, (X) ⟶ (VIII)] which operates generally, but not exclusively, when alcoholic hydrogen chloride is used as a reagent on 2-hydroxyalkylamides (Phillips and Baltzly, *loc. cit.*; Welsh, *J. Amer. Chem. Soc.*, 1949, **71**, 3500; Elliott, *Biochem. J.*, 1952, **50**, 542). It is possible that the absence of steric hindrance at the reactive centre encouraged cyclisation by this mechanism. This would explain the fact that the dithiocarbamic esters derived from the substituted ethanolamines gave poor yields of the corresponding thiazolines under the influence of alcoholic hydrogen chloride. In addition to the derivative of dithiolcarbonic acid, already described, a certain amount of 2-chloro-1-thiolcarbobenzyloxyaminopropane (XI) was isolated after treatment of the crude reaction product from (I; R = Me, R′ = H, R″ = $CH_2Ph$) and thionyl chloride with boiling 6N-hydrochloric acid. It is difficult to visualise the formation of this substance from a thiazoline, and it is concluded that the crude product from the reaction with thionyl chloride contained some oxazoline which was converted into the chloro-compound on treatment with hydrochloric acid. The following reaction scheme, which is in accordance with the above ideas, is suggested :



The possibility that the chloro-compound (XI) was produced directly from the dithiocarbamate is excluded by the fact that the crude product from the thionyl chloride reaction was insoluble in ether whereas (XI) was easily soluble. Unfortunately, all attempts to isolate the oxazoline in a pure state were unavailing. The stability of the chloro-compound towards acid is in keeping with Ehrensvärd's observations (*Nature*, 1947, **159**, 500) on this class of compound. The presence of some oxazoline was also demonstrated in the product resulting from the cyclisation of 3-*N*-dithiocarbobenzyloxyaminobutan-2-ol (I; R = R′ = Me, R″ = $CH_2Ph$) with thionyl chloride. Samples of the thiazoline (II; R = R′ = Me, R″ = $CH_2Ph$) which had been distilled only once yielded on hydrolysis with 6N-hydrochloric acid a mixture of 2-amino-1-methyl-*n*-propyl *S*-benzyl thiolcarbonate hydrochloride (XII) and the dithiolcarbonate hydrochloride (VI; R = R′ = Me, R″ = $CH_2Ph$). The properties of the hydrochloride (XII) were similar to those of the dithiolcarbonic acid

derivatives (VI); it was very resistant to acid hydrolysis and yielded toluene-ω-thiol on treatment with alcoholic triethylamine.

After this work was commenced a publication by Niederl and Hart (*loc. cit.*) came to our notice. As a result of previous researches by Niederl and his collaborators on the condensation of mustard oils with phenols these workers believed that they had obtained arylthio-$\Delta^2$-thiazolines by the condensation of allyl *iso*thiocyanate with thiophenols in the presence of hydrogen chloride:




Niederl and Hart's compounds, however, did not have the properties expected of arylthio-$\Delta^2$-thiazolines; they were stated to be very stable to acid treatment, but were completely hydrolysed in dilute sodium hydrogen carbonate solution, giving a thiophenol and 2-keto-5-methylthiazolidine. These properties were displayed by our dithiolcarbonic acid derivatives (VI), but not by the thiazolines from which they were derived. Niederl and Hart's compounds were thus probably dithiolcarbonic acid derivatives, the more so as the condensation products were dissolved in water and the acid solution evaporated to dryness on the water-bath: our work showed that such treatment would have decomposed the arylthiothiazoline initially formed. Condensation of allyl *iso*thiocyanate with thiophenol under Niederl and Hart's conditions (*loc. cit.*) was therefore repeated, precautions being taken to exclude moisture. After a considerable time a portion of the reaction mixture, which was still liquid, was removed and after a preliminary extraction with ether was treated at once with an excess of sodium picrate. The crystalline picrate so obtained had a melting point different from that of the compound described by Niederl and Hart; it was undoubtedly the picrate of 5-methyl-2-phenylthio-$\Delta^2$-thiazoline which Niederl and Hart believed they had prepared. The free base, obtained from the picrate, was purified by distillation and found to be completely resistant to the action of sodium hydrogen carbonate solution, but it was decomposed by boiling hydrochloric acid, yielding 1-amino-2-propyl *S*-phenyl thiolcarbonate hydrochloride. This substance was found to have the same melting point as the hydrochloride described by Niederl and Hart and was decomposed immediately by sodium hydrogen carbonate solution or by alcoholic triethylamine. In view of our work it is improbable that any of the compounds described by Niederl and Hart were arylthiothiazolines, although thiazolines were produced initially and could have been isolated had these workers been aware of their instability.

## Experimental

*Amino-alcohols.*—2-Aminopropan-1-ol, 1-aminopropan-2-ol, and 3-aminobutan-2-ol were prepared from the corresponding nitro-alcohols (Vanderbilt and Haas, *Ind. Eng. Chem.*, 1940, **32**, 34) by hydrogenation at room temperature/100 atm., Adams's platinum oxide catalyst being used. The resulting amino-alcohols were isolated and purified by crystallisation of their oxalates from ethyl alcohol. No attempt was made to separate the diastereoisomeric forms of 3-aminobutan-2-ol.

2-N-*Dithiocarbobenzyloxyaminoethanol.*—To ethanolamine (10·0 g.) in pyridine (30 ml.), triethylamine (1 mol., 16·6 g.) and carbon disulphide (1·1 mols., 13·7 g.) were added with water-cooling. Considerable heat was evolved. The solution was then cooled to 0° and kept at this temperature for 1 hour; benzyl chloride (1 mol., 20·8 g.) was added, and the solution kept at 0° overnight. The solution was then poured into 3N-sulphuric acid (800 ml.) and extracted with ether. The ethereal extract was washed with 3N-sulphuric acid and sodium hydrogen carbonate solution and dried ($Na_2SO_4$). After evaporation of the ether, light petroleum (100 ml.; b. p. 40—60°) was added to the residual oil, which rapidly crystallised; the solid (33 g., 90%) had m. p. 55°. Repeated recrystallisation from benzene gave pure 2-N-*dithiocarbobenzyloxyaminoethanol*, m. p. 64° (Found: C, 52·8; H, 5·3; N, 6·3. $C_{10}H_{13}ONS_2$ requires C, 52·9; H, 5·7; N, 6·2%).

2-N-*Dithiocarbobenzyloxyaminopropan*-1-*ol.*—2-Aminopropan-1-ol oxalate (m. p. 127—128°; 20 g.) was suspended in pyridine (90 ml.), triethylamine (50 g., 4 mol.) was added, and the mixture vigorously shaken to disperse any large particles. After 1 hour this mixture was

cooled to 0°, and carbon disulphide (2 mols., 19 g.) was added during 15 minutes with shaking, the temperature being kept below 5°. After 2 hours at 0°, benzyl chloride (1 mol., 16 g.) was added, and the mixture left at 0° overnight. The reaction mixture was then treated as above, and the crude product isolated as an oil (26·7 g., 89%).

1-N-*Dithiocarbobenzyloxyaminopropan-2-ol*.—This was prepared in the same manner as its isomer. 1-Amino-2-propan-1-ol oxalate (m. p. 126°; 20 g.) gave 23·5 g. (79%) of 1-N-*dithiocarbobenzyloxyaminopropan-2-ol*, which was an oil (Found: S, 27·0. $C_{11}H_{15}ONS_2$ requires S, 26·6%).

3-N-*Dithiocarbobenzyloxyaminobutan-2-ol*.—This was prepared both from 3-aminobutan-2-ol and from its oxalate (m. p. 164°) in 94% yield. The *product* was an oil (Found: C, 56·6; H, 7·6; N, 5·3. $C_{12}H_{17}ONS_2$ requires C, 56·5; H, 6·7; N, 5·5%).

*Cyclisation of the Dithiocarbamates to* $\Delta^2$-*Thiazolines*.—2-*Benzylthio*-$\Delta^2$-*thiazoline*. 2-*N*-Dithiocarbobenzyloxyaminoethanol (10 g.) was added during 15 minutes to thionyl chloride (20 ml., purified as described in *Org. Synth.*, Coll. Vol. II, p. 570) cooled in ice, and the reaction mixture kept at 0° for 1 hour. Excess of thionyl chloride was removed under reduced pressure at 35°. Anhydrous ether (50 ml.) was added to the residual oil, which crystallised when scratched, giving 7·59 g. (82%) of solid, m. p. 130°. Recrystallisation from chloroform–light petroleum (b. p. 40—60°) gave pure 2-*benzylthio*-$\Delta^2$-*thiazoline hydrochloride*, m. p. 138° (Found: Cl, 14·6. $C_{10}H_{12}NClS_2$ requires Cl, 14·5%). To prepare the free base, the hydrochloride was covered with a layer of ether, and excess of sodium hydrogen carbonate solution was added. The ethereal layer was dried ($Na_2SO_4$) and evaporated. The residual oil was distilled, to give 2-*benzylthio*-$\Delta^2$-*thiazoline*, b. p. 130°/0·05 mm., m. p. 47° (Found: N, 6·9; S, 29·9. $C_{10}H_{11}NS_2$ requires N, 6·7; S, 30·7%).

2-*Benzylthio*-5-*methyl*-$\Delta^2$-*thiazoline*. 1-*N*-Dithiocarbobenzyloxyaminopropan-2-ol (20·7 g.) in anhydrous ether (15 ml.) was added in small portions to purified thionyl chloride (60 ml.) cooled in ice. After 2 hours at 0°, the thionyl chloride was removed under reduced pressure at 30°. The resulting oil, which was completely insoluble in anhydrous ether, was poured into a saturated aqueous solution of sodium hydrogen carbonate, and the free base isolated by ether. The crude product (13·9 g., 73%) was purified by distillation (b. p. 135°/1 mm., 125°/0·5 mm.) or as its *picrate*, m. p. 161° (Found: C, 45·8; H, 3·5; N, 12·4. $C_{17}H_{16}O_7N_4S_2$ requires C, 45·1; H, 3·5; N, 12·4%). Both these methods involved large losses, probably because the crude oil contained compounds other than the thiazoline.

Crude 2-*N*-dithiocarbobenzyloxyaminopropan-1-ol (26·7 g.) was cyclised in a similar fashion, and the crude thiazoline (22 g., 89%) converted by ethereal hydrogen chloride into 2-*benzylthio*-4-*methyl*-$\Delta^2$-*thiazoline hydrochloride*, m. p. 80° after crystallisation from chloroform–ether (Found: C, 50·7; H, 5·8; N, 5·5. $C_{11}H_{14}NClS_2$ requires C, 50·9; H, 5·4; N, 5·4%).

2-*Benzylthio*-4: 5-*dimethyl*-$\Delta^2$-*thiazoline*, similarly prepared, had b. p. 118°/0·05 mm. (Found: C, 60·6; H, 6·7; N, 5·9. $C_{12}H_{15}NS_2$ requires C, 60·8; H, 6·3; N, 5·9%). The *picrate* had m. p. 128° (Found: N, 12·0. $C_{18}H_{18}O_7N_4S_2$ requires N, 12·0%).

The corresponding 2-methylthio-$\Delta^2$-thiazolines were prepared from 2-*N*-dithiocarbomethoxyaminoethanol and its homologues, which were used in the crude state. 2-Methylthio-$\Delta^2$-thiazoline had b. p. 46°/14 mm. (Gabriel, *Ber.*, 1889, **22**, 1153, gives b. p. 216—217°/760 mm.) (Found: N, 10·5. Calc. for $C_4H_7NS_2$: N, 10·5%). The *picrate* had m. p. 123° (Found: N, 15·5. $C_{10}H_{10}O_7N_4S_2$ requires N, 15·5%). 5-*Methyl*-2-*methylthio*-$\Delta^2$-*thiazoline picrate* had m. p. 122° (Found: N, 14·5; S, 16·2. $C_{11}H_{12}O_7N_4S_2$ requires N, 14·9; S, 17·0%). The free base has been prepared previously by Hirsch (*Ber.*, 1890, **23**, 967). 4: 5-*Dimethyl*-2-*methylthio*-$\Delta^2$-*thiazoline picrate* had m. p. 121° (Found: N, 14·6. $C_{12}H_{14}O_7N_4S_2$ requires N, 14·4%).

*Alternative Methods of Cyclisation of* 2-N-*Dithiocarbobenzyloxyaminoethanol*.—(*a*) 2-*N*-Dithiocarbobenzyloxyaminoethanol (0·5 g.) was heated in a 3N-solution of hydrogen chloride in anhydrous methyl alcohol (6 ml.) at 105° for 6 hours. The solution was then evaporated, leaving a crystalline residue (0·4 g.), m. p. 164°, raised by recrystallisation from acetic acid to 175°. This substance was identical with an authentic specimen of 2-aminoethyl *S*-benzyl thiolcarbonate hydrochloride.

(*b*) 2-*N*-Dithiocarbobenzyloxyaminoethanol (3 g.) was dissolved in anhydrous ethyl alcohol at 0° which had previously been saturated with anhydrous hydrogen chloride. This solution was kept at 0° overnight, and the alcohol distilled off under reduced pressure. A crystalline solid (2·8 g.), m. p. 138°, remained, identical with 2-benzylthiothiazoline hydrochloride prepared by the thionyl chloride cyclisation.

(*c*) 2-*N*-Dithiocarbobenzyloxyaminoethanol (3 g.) was dissolved in anhydrous ether (10 ml.), and phosphorus tribromide (1·1 mols.; 4 g.) was added dropwise. A vigorous reaction occurred with precipitation of a white solid. After 30 minutes the solid was filtered off and dried in a

vacuum-desiccator over sodium hydroxide. The yield was 3·92 g. (theoretical yield for 2-benzylthiothiazoline hydrobromide is only 3·8 g.), m. p. 164° (Found: Br, 25·4. Calc. for $C_{10}H_{12}NBrS_2$: Br, 27·6%). It is probable that the discrepancies in yield and analysis were the result of contamination by phosphorus. The hydrobromide was converted into the hydrochloride, which had m. p. 134°.

*2-Aminoethyl Benzyl Dithiolcarbonate Hydrochloride.*—2-Benzylthiothiazoline hydrochloride (12 g.) was heated under reflux with 6N-hydrochloric acid (120 ml.) for 4 hours, the solution was cooled at 0° for 1 hour, and the crystalline solid (11·35 g.; m. p. 175°) filtered off. Recrystallisation from acetic acid gave pure *2-aminoethyl benzyl dithiolcarbonate hydrochloride*, m. p. 179° (Found: C, 45·2; H, 5·0; N, 5·4; Cl, 13·7. $C_{10}H_{14}ONClS_2$ requires C, 45·6; H, 5·3; N, 5·3; Cl, 13·5%). The formyl derivative was prepared by the method used by Crawhall and Elliott (*loc. cit.*) for this type of compound and had m. p. 94°.

*2-Acetamidoethyl Benzyl Dithiolcarbonate.*—The foregoing dithiolcarbonate hydrochloride (0·26 g.) was dissolved in acetic anhydride (1·5 ml.), hydrated sodium acetate (1 mol., 0·13 g.) was added, and the solution heated on a steam-bath for 30 minutes, then filtered hot and evaporated under reduced pressure, leaving a clear oil (0·22 g., 83%) which crystallised on addition of light petroleum (b. p. 40—60°). The *acetyl* derivative, recrystallised from ligroin–benzene (9 : 1), had m. p. 83° (Found: C, 53·7; H, 5·5; N, 5·3. $C_{12}H_{15}ONS_2$ requires C, 53·5; H, 5·6; N, 5·2%).

*2-Aminopropyl Benzyl Dithiolcarbonate Hydrochloride.*—Crude 2-benzylthio-4-methylthiazoline (22 g.) was heated under reflux with 6N-hydrochloric acid (200 ml.) for 6 hours. The solution was allowed to cool to room temperature, and the aqueous layer decanted from the lower layer of oil. The acid layer was left overnight at 0°. A white crystalline solid (2·24 g.) appeared. This was the *dithiolcarbonate hydrochloride*, m. p. 122—123° (Found: C, 47·0; H, 5·8; N, 4·8. $C_{11}H_{16}ONClS_2$ requires C, 47·7; H, 5·8; N, 5·0%). The oil resulting from the hydrolysis was added to water (200 ml.), and the solution extracted with ether (200 ml.). The aqueous layer was evaporated to small bulk, yielding an additional crop (12·63 g.) of the dithiolcarbonic acid derivative which had m. p. 122° after two crystallisations from ethyl acetate.

*2-Aminoethyl Methyl Dithiolcarbonate Hydrochloride.*—This was obtained from the hydrolysis of 2-methylthiothiazoline by 6N-hydrochloric acid in the same manner as the corresponding benzyl derivative. Recrystallisation from acetic acid gave the pure *dithiolcarbonate hydrochloride*, m. p. 156° (Found: N, 7·5. $C_4H_{10}ONClS_2$ requires N, 7·5%).

*1-Amino-2-propyl Benzyl Dithiolcarbonate Hydrochloride.*—(*a*) 2-Benzylthio-5-methylthiazoline picrate (3·5 g.) was suspended in water (20 ml.), and the free thiazoline liberated by addition of ethanolamine (2 mol., 0·47 g.) and thorough shaking according to the method of Kaye, Kogon, and Burbont (*J. Amer. Chem. Soc.*, 1950, **72**, 5752). The thiazoline was isolated by ether-extraction, evaporation, and extraction from the last traces of picric acid with light petroleum (b. p. 40—60°). The clear oil (1·58 g.) was heated under a reflux condenser with 6N-hydrochloric acid (15 ml.) for 6 hours. After cooling, the solution was made homogeneous by addition of ethyl alcohol to a total volume of 30 ml. An aliquot (1·5 ml.) was directly titrated by iodine solution and gave a very small value, but when another aliquot (1·5 ml.) was first basified with 3N-sodium hydroxide, kept for 2 minutes in a stoppered flask, acidified with concentrated hydrochloric acid, and titrated with iodine, 86% of the theoretical amount was consumed. This iodine consumption was due to the formation of a ketothiazolidine from the dithiolcarbonic acid derivative under the influence of alkali with liberation of toluene-ω-thiol, and shows that at least 86% of the dithiolcarbonate had been formed. The remainder of the alcoholic solution was evaporated to dryness, and the crystalline residue leached with acetone. A further crop of solid was obtained by addition of ether to the acetone washings. The combined solids (1·03 g., 53%) were crystallised from 6N-hydrochloric acid to give a product, m. p. 123°, identical with that described below.

(*b*) Crude 2-benzylthio-5-methylthiazoline (13·92 g.) was heated under reflux with 6N-hydrochloric acid (100 ml.) for 6 hours. After cooling, the acid solution was decanted from the lower layer of oil, which was then dissolved in ether; the solution was dried, and light petroleum added, giving a crystalline solid, m. p. 60—62° (1·0 g.). Crystallisation from light petroleum (b. p. 40—60°) raised the m. p. to 64° (Found: C, 54·8; H, 5·85; N, 5·3; Cl, 14·4; S, 13·7. $C_{11}H_{14}ONClS$ requires C, 54·4; H, 5·75; N, 5·7; Cl, 14·6; S, 13·2%). This compound was formulated as *2-chloro-1-thiolcarbobenzyloxyaminopropane* because it contained non-ionic chlorine and liberated toluene-ω-thiol on treatment with caustic alkali, but gave none with sodium hydrogen carbonate or with an alcoholic solution of triethylamine.

The acid solution was evaporated, and the residual oil dried in a vacuum-desiccator. It was then dissolved in an equal volume of glacial acetic acid, and anhydrous ether added, giving a precipitate of *1-amino-2-propyl benzyl dithiolcarbonate hydrochloride* (3·04 g.), m. p. 123—124°, which had m. p. 124° after crystallisation from 6N-hydrochloric acid (Found: C, 47·6; H, 5·7; N, 5·1. $C_{11}H_{14}ONClS_2$ requires C, 47·7; H, 5·8; N, 5·05%).

*2-Amino-1-methylpropyl Benzyl Dithiolcarbonate Hydrochloride.*—2-Benzylthio-4 : 5-dimethylthiazoline (3·02 g.) which had been distilled once and was still impure was heated under reflux with 6N-hydrochloric acid (30 ml.) for 6 hours. On cooling, the *dithiolcarbonate hydrochloride* crystallised (0·7 g.), m. p. 170—174°. The acid was evaporated off, the residual oil dissolved in a small volume of ethanol and, on addition of ether, a second crop (0·52 g.), m. p. 159—165°, was obtained. On careful recrystallisation from 6N-hydrochloric acid the m. p. of both crops was raised to 174°. The pure substance crystallised in elongated rectangular plates (Found: C, 49·4; H, 6·8; N, 4·8. $C_{12}H_{18}ONClS_2$ requires C, 49·5; H, 6·2; N, 4·8%). The alcohol–ether filtrate from the above crystallisation was evaporated, leaving a clear oil (1·72 g.). This was dissolved in acetone, and on addition of successive quantities of ether two further crops, m. p. 90—110° (0·68 g.) and m. p. 80—95° (0·21 g.), were obtained. After isolation, this material was insoluble in acetone and was repeatedly crystallised from 6N-hydrochloric acid, forming flat plates, m. p. 140°. This lower-melting material liberated toluene-ω-thiol after treatment with alcoholic triethylamine, contained ionic chlorine, and is formulated as *2-amino-1-methylpropyl S-benzyl thiolcarbonate hydrochloride* (Found: C, 52·4; H, 6·4; N, 4·9; S, 11·7. $C_{12}H_{18}O_2NClS$ requires C, 52·4; H, 6·5; N, 5·1; S, 11·6%).

The refractive indices of the crystals of the two substances were measured by an immersion method using the polarising microscope. The butane-2-thiol derivative had $n^{20}$ 1·56 and 1·70, and the butan-2-ol derivative 1·555 and 1·61. When the latter substance was ground, no particles of $n^{20}$ 1·70 could be found.

*Conversion of the Dithiolcarbonates into 2-Ketothiazolidines.*—*2-Ketothiazolidine.* 2-Aminoethyl benzyl dithiolcarbonate hydrochloride (1 g.) was suspended in ethyl alcohol (45 ml.) and N-sodium hydroxide (3 mols., 11·4 ml.) was added, giving a total volume of 58 ml., and the mixture was kept in a stoppered flask for 3 hours. A portion (5 ml.) was then acidified with hydrochloric acid, and the liberated toluene-ω-thiol titrated with 0·1N-iodine; 2·7 ml. were required, representing 87% of the theoretical amount. The remaining solution was neutralised with hydrochloric acid, and the solvent distilled off. The residue was thoroughly extracted with ether, and the ethereal extract evaporated to dryness, leaving a crystalline residue; the yield was 0·33 g. (98%), m. p. 47—49°. Recrystallisation from ether gave pure *2-ketothiazolidine*, m. p. 54°, b. p. 160°/20 mm. (Found: C, 34·8; H, 4·7; N, 13·6. $C_3H_5ONS$ requires C, 35·0; H, 4·8; N, 13·6%). This reaction also occurred in the presence of sodium hydrogen carbonate, but for preparative purposes it was more convenient to use sodium hydroxide. The thiazolidine was obtained in a similar way from the corresponding methyl ester hydrochloride.

*2-Keto-4-methylthiazolidine*, b. p. 165°/10 mm. (Found: C, 41·8; H, 6·2; N, 11·8. $C_4H_7ONS$ requires C, 41·0; H, 6·0; N, 12·0%), and *2-keto-5-methylthiazolidine*, b. p. 140°/10 mm. (Found: C, 41·5; H, 5·8; N, 12·1%), were prepared similarly, except that alcohol instead of ether was used to extract the crude thiazolidines from sodium chloride.

*2-Keto-4 : 5-dimethylthiazolidine.*—2-Amino-1-methylpropyl benzyl dithiolcarbonate hydrochloride (2 g.) was shaken in a stoppered flask with N-sodium hydroxide (21 ml., 3 mol.) for 5 minutes, N-hydrochloric acid (24 ml.) was added to the resulting emulsion, and the solution extracted with light petroleum (b. p. 60—80°; 3 × 25 ml.). The aqueous layer was evaporated to dryness under reduced pressure, and the residue extracted with ether (3 × 20 ml.). The extracts were combined and evaporated, and the residual oil was crystallised from ether–light petroleum (b. p. 40—60°). *2-Keto-4 : 5-dimethylthiazolidine* had m. p. 47° (Found: C, 46·4; H, 7·1; N, 10·6; S, 24·6. $C_5H_9ONS$ requires C, 45·8; H, 6·9; N, 10·6; S, 24·5%).

*N-Benzoylcysteine.*—2-Phenylthiazoline-4-carboxylic acid (2·0 g.) was added to boiling distilled water (100 ml.) under a reflux condenser in an atmosphere of nitrogen. After 15 minutes an aliquot (2·0 ml.) was removed and, after cooling in ice and acidification with dilute hydrochloric acid, was found to consume the amount of iodine expected if complete conversion into *N*-benzoylcysteine had occurred. That the acidification before titration was not responsible for opening the ring was shown by titration of an aliquot which was not acidified. Somewhat more than the theoretical amount was consumed. This is in accordance with Lavine's observation (*J. Biol. Chem.*, 1935, **109**, 141) that titration of cysteine must be conducted in the presence of mineral acid. After cooling of the main solution to 0° overnight, *N*-benzoylcysteine (1·59 g.), m. p. 137° (Fry, *J. Org. Chem.*, 1950, **15**, 439, gives m. p. 136—137°), was filtered off (Found:

N, 6·2. Calc. for $C_{10}H_{11}O_4NS$ : N, 6·2%). A second crop (0·32 g.) was obtained by evaporation of the mother-liquors.

*Investigation of the Structure of Some Compounds described by Niederl and Hart.*—*5-Methyl-2-phenylthio-$\Delta^2$-thiazoline.* Allyl *iso*thiocyanate (9·9 g.) was mixed with thiophenol (1 mol., 11·0 g.) in a crystallising dish in a vacuum-desiccator. The desiccator was evacuated and then filled with anhydrous hydrogen chloride. This process was repeated at intervals of a few days until no more hydrogen chloride appeared to be absorbed. The mixture was then kept in the presence of hydrogen chloride for 2½ months. Even after this time no crystallisation took place. Water was added to a portion of the reaction mixture and after extraction with ether the aqueous layer was treated with a solution of sodium picrate. The precipitate was filtered off, washed with ethanol, and crystallised from ethanol, to give long needles of *5-methyl-2-phenylthio-$\Delta^2$-thiazoline picrate*, m. p. 132° (Found : C, 44·2; H, 3·3; N, 12·9. $C_{16}H_{14}O_7N_4S_2$ requires C, 43·8; H, 3·2; N, 12·9%). The picrate was decomposed with ethanolamine in the usual way, to give an oil which was purified by distillation in a short-path vacuum apparatus at 120° (bath-temp.)/0·1 mm., to give pure *5-methyl-2-phenylthio-$\Delta^2$-thiazoline* (Found : C, 57·4; H, 5·3; N, 6·9; S, 29·8. $C_{10}H_{11}NS_2$ requires C, 57·4; H, 5·3; N, 6·7; S, 30·7%). The thiazoline gave no thiophenol, as revealed by iodine titration when kept for 1 hour with excess of sodium hydrogen carbonate solution. The pure thiazoline did not give a crystalline hydrochloride.

*1-Amino-2-propyl phenyl dithiolcarbonate hydrochloride.* (*a*) A portion of the original reaction mixture was boiled with water for 30 minutes and, after cooling, the crystalline precipitate was filtered off and leached with ethanol. Crystallisation from ethanol gave the *dithiolcarbonate hydrochloride*, m. p. 168° (Found : C, 45·6; H, 5·4; N, 5·4. $C_{10}H_{14}ONClS_2$ requires C, 45·7; H, 5·3; N, 5·3%). Niederl and Hart (*loc. cit.*) gave m. p. 171° for the compound which they believed to be 5-methyl-2-phenylthio-$\Delta^2$-thiazoline hydrochloride.

(*b*) Pure 5-methyl-2-phenylthio-$\Delta^2$-thiazoline was heated under a reflux condenser with 6N-hydrochloric acid for 30 minutes. The solution was evaporated, and the residual oil crystallised by addition of ether to its solution in ethanol. The product had m. p. 166° undepressed on admixture with the previously prepared sample.

The remaining crude reaction mixture, when kept in the presence of moisture, slowly solidified. After 12 months the product was found to be the dithiolcarbonate hydrochloride, m. p. 167°.

*Action of alkali.* The hydrochloride (0·0252 g.) was suspended in 1% aqueous sodium hydrogen carbonate solution (2 ml.) and the mixture kept for 30 minutes in a stoppered flask. The solution was then acidified with 3N-hydrochloric acid. Titration with iodine revealed that 0·85 mol. of thiophenol had been liberated. A similar result was obtained when the hydrochloride was treated with an alcoholic solution of triethylamine.

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## 594. *Anthelmintics: Kousso. Part II.* The Structures of Protokosin, α-Kosin, and β-Kosin.*

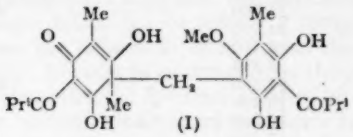
By A. J. Birch and A. R. Todd.

New formulæ are proposed for protokosin (I, or a tautomer) and α- (II) and β-kosin (III) which are based chiefly on absorption spectra and reduction of the kosins with sodium amalgam to methylenebismethylphloroglucinol derivatives and *iso*butaldehyde.

The isolation of protokosin from kousso (*Hagenia abyssinica*) flowers was described in Part I,* together with some degradative studies on the compound. The available evidence was sufficient to indicate that protokosin was related in structure to some of the methylenebisphloroglucinol derivatives, *e.g.*, aspidin (V), isolated by Boehm from *Aspidium filix mas*

* Part I, Hems and Todd, *J.*, 1937, 562.

(cf., *e.g.*, *Annalen*, 1903, **329**, 321), and for it a formula $C_{22}H_{28}O_7$ (IV) was suggested as a basis for further work. Further investigation of protokosin has been severely hampered by lack of material. The original source was a commerical sample of kousso of unknown origin. More recently, through the kind co-operation of the Colonial Products Research Council who arranged for plant collection, authentic samples of dried flowers, male and female, of *Hagenia abyssinica* have been obtained from various localities in East Africa; from only one of these was it possible to isolate crystalline protokosin and even there the yield was very low, although extracts of the flowers contained large amounts of amorphous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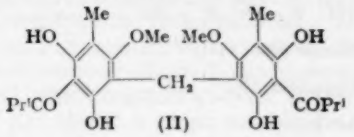
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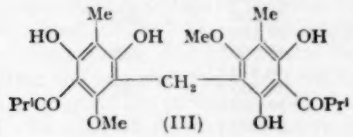
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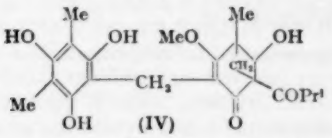
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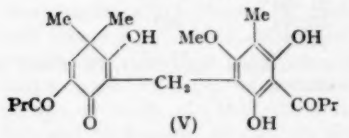
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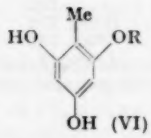
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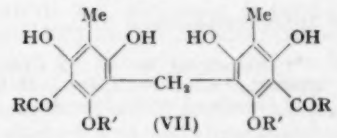
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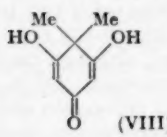
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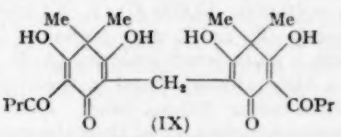
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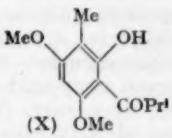

(X)

material some of which could be converted into kosins by the action of alkali (Rivett, Thesis, Cambridge 1948). Studies of these complex materials are as yet very incomplete, but we have recently re-examined the known facts about protokosin in the light of some further experimental results and have reached certain conclusions which are now presented.

The published evidence relating to the structure of protokosin can be summarised as follows. On reduction with zinc dust and sodium hydroxide it gave rise to *iso*butyric acid and a mixture of methylphloroglucinols from which was isolated 1 : 3 : 5-trimethylphloroglucinol, together with the compounds α- and β-kosin which are isomeric with the starting material but possess two methoxyl groups instead of one. Protokosin gave rise on alkali-fusion to methylphloroglucinol (VI; R = H), and it had been observed by Lobeck (*Arch. Pharm.*, 1901, **239**, 672) that α-kosin on vigorous treatment with alkali or acid produced some methylphloroglucinol β-methyl ether (VI; R = Me). Protokosin is optically active and the kosins are inactive. It was considered that the latter are derived from the former by migration of a methyl group from carbon to oxygen. We now consider that the molecular formula of protokosin should be revised to $C_{25}H_{32}O_8$ and that the most adequate structures are (I) for protokosin, (II) for α-kosin, and (III) for β-kosin.

The suggestion that a considerable alteration might have to be made to (IV) was first put forward by Mulholland (Thesis, Manchester, 1947) who pointed out that the ultra-violet absorption spectra of protokosin and the kosins could not be reconciled on the basis of (IV) with those of a number of model compounds. The absorptions of some relevant compounds are given in the Table.

It is apparent that compounds containing an acylphloroglucinol nucleus possess between 200 and 300 mμ a maximum at 223—233 mμ and another at 271—293 mμ, the former having a greater regularity of position and intensity. The intensity at the shorter wave-length in

| | $\lambda_{max.}$ (mμ) | $\varepsilon_{max.}$ | $\lambda_{min.}$ (mμ) | Ref. |
|---|---|---|---|---|
| A, Methylphloroglucinol | 270·5 | 623 | 254 | 1 |
| B, Dimethylphloroglucinol | 277 | 1,070 | 254 | 1 |
| C, Trimethylphloroglucinol | 271 | 900 | — | 2 |
| | 274 | 885 | | |
| D, Phloro*iso*butyrophenone | 229·3 | 12,800 | 247·5 | 1 |
| | 289 | 16,000 | | |
| E, *C*-Acetyldimedon | 231 | 10,000 | 250 | 3 |
| | 277 | 10,000 | | |
| F, (VII; R = Me, R′ = H) | 227 | 21,800 | — | 1 |
| | 291 | 25,760 | | |
| G, (VII; R = Pr$^{i}$, R′ = Me) | 233 | 27,700 | — | 4 |
| | 292·5 | 24,700 | | |
| H, Filicinic acid (VIII) | 244 | 15,100 | — | 4 |
| | 283 | 4,350 | | |
| J, Albaspidin (IX) | 223 | 27,300 | — | 4 |
| | 271 | 13,400 | | |
| K, Baeckeol (X) | 227 | 10,300 | 250 | 1 |
| | 293 | 17,300 | | |
| L, Flavaspidic acid | 229 | 26,350 | 255 | 1 |
| | 292·5 | 20,300 | | |
| M, Protokosin | 223 | 25,860 † | 253 | |
| | 287 | 19,840 † | | |
| N, α-Kosin | 230 | 30,000 † | 254 | 1 |
| | 287 | 23,000 † | | |
| O, β-Kosin | 228 | 30,300 † | 254·5 | 1 |
| | 292 | 21,260 † | | |
| P, Methylenebisbaeckeol | Inflex. 232 | 23,880 | 250 | |
| | Max. 286 | 28,000 | | |

1, Mulholland, *loc. cit.*; 2, Campbell and Coppinger, *J. Amer. Chem. Soc.*, 1951, **73**, 2708; 3, Birch, *J.*, 1951, 3026; 4, Dr. T. H. Quibell, personal communication.
† Calculated on $C_{25}$ formula.

compounds with one nucleus is 10,000—13,000 (D, E, K) and in those with two nuclei insulated by a methylene group (F, G, J, and probably L) is 22,000—27,000. An unacylated nucleus absorbs with a much lower intensity (A, B, C), and, although the non-aromatic compound (H) has a high-intensity band in the region concerned, this is at a higher wave-length (244 mμ) than the *ca.* 230-mμ band. Evidently, if protokosin and the kosins (M, N, O) have two acylphloroglucinol nuclei their absorption spectra possess maxima of about the right positions and intensities. It is difficult to reconcile their spectra with a formula (IV) containing one unacylated nucleus. The two bands seem to be characteristic of the enolised β-triketone system and they are found even with the non-aromatic compounds (E, J); the triketone system in (IV) cannot enolise because of the bridge.

Another observation which is not in accord with (IV) is the production of two mols. of *iso*butyric acid on hydrolysis with sulphuric acid (Leichsenring, *Arch. Pharm.*, 1890, **232**, 50; Rivett, *loc. cit.*) although the fact that the acid was estimated merely by titration might permit of other explanations. The only likely formula which fits the analytical figures and allows the presence of two *iso*butyrylmethylphloroglucinol nuclei is $C_{25}H_{32}O_8$ [Found, for protokosin: C, 65·4, 65·2, 65·4; H, 7·0, 7·0, 7·0; MeO, 8·0, 8·1; 8·1%; *M* (Rast), 412, 396, 399. Found, for α-kosin: C, 65·1, 65·3; H, 7·0, 6·8; MeO, 13·2, 13·5. Found, for β-kosin: C, 65·3; H, 7·0; MeO, 13·4%; *M*, 460 (Hems and Todd, *loc. cit.*). Calc. for $C_{25}H_{32}O_8$: C, 65·2; H, 7·0; MeO, 6·8; 2MeO, 13·4%; *M*, 460]. The molecular-weight determinations are about 15% low for the $C_{25}$-formula, but this error is not exceptional for Rast determinations. The methoxyl value of 8% for protokosin is high for one methoxyl group (6·8%), but some isomerisation to kosin may occur in the course of the determination; furthermore the considerably different figure of 10·4% was found by other analysts. Recalculation of the *C*-Me figures (Hems and Todd, *loc. cit.*) for the $C_{25}$-formula gives maximum values of just over 3 for β-kosin and 3·7 for protokosin, indicating that these compounds may well contain the 4 and 5 *C*-Me groups to be expected on the assumption that an *iso*butyryl group is estimated as one *C*-Me. The fact that the figure for protokosin is significantly higher than for β-kosin supports the postulated migration from carbon to oxygen during the formation of the latter.

The fully methylated kosins, obtained by exhaustive methylation with methyl sulphate

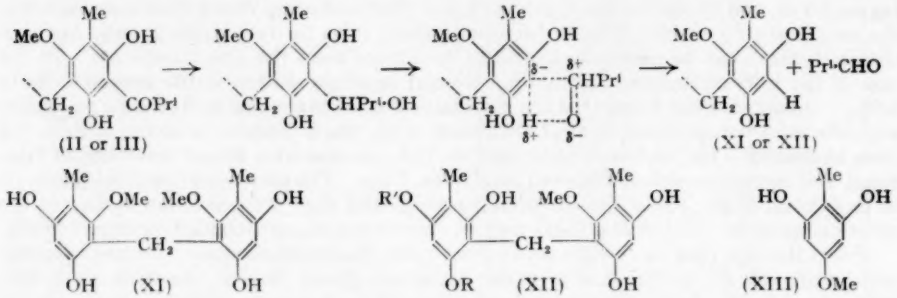
and sodium hydroxide, have now been shown to be identical by means of their infra-red spectra. The difference between the kosins therefore results from differing positions of the methylated hydroxyl groups; this is further confirmed by the identity of the fully methylated reduction products described below.

The infra-red spectra of protokosin and the kosins (see Experimental section) show a strong likeness, particularly the latter compounds, and possess bands due to (probably) hydrogen-bonded carbonyl (6·2 μ), hydrogen-bonded hydroxyl (general absorption in the region 3·3 μ), and normal hydroyl groups (3 μ). This is the first direct demonstration that the substances do, in fact, contain carbonyl groups, since no derivatives have so far been obtained which can be definitely identified as derived from the intact molecule. In the case of the fully methylated kosins the carbonyl band has shifted to the normal value of 5·85 μ. In order to be certain that the postulated non-aromatic ring in (I) can be reconciled with the infra-red spectrum, that of albaspidin (IX), which contains a similar system, has been examined. The carbonyl band here at 6·13 μ is also at a longer wave-length than usual, and there is no sign of a normal band at *ca.* 5·9 μ. The presence of two OH-bands (?) in protokosin (2·96, 3·23 μ) is somewhat puzzling, and they are not found in any of the other compounds. The 3·23 μ band may be due to a hydrogen-bonded hydroxyl group.

From the fact that the kosins fail to react with diazoaminobenzene, one and possibly both methoxyls are probably *ortho* to the methylene group (Boehm, *Annalen*, 1903, **329**, 301). In order to obtain more information it would be necessary either to synthesise the compounds, which should be possible by standard methods (*e.g.*, McGookin, Robertson, and Simpson, *J.*, 1951, 2021), or else to convert them into more readily synthesised compounds. Sodium amalgam seemed a possible reagent for this purpose, because its action might be expected to lead to fission of the molecule, or removal of the *iso*butyryl groups, or both. In fact, the action of 3% sodium amalgam on a cold alkaline solution, followed by heating, resulted in the formation of *iso*butaldehyde and methylenebismethylphloroglucinol derivatives in fair yield. The fission is evidently a reverse aldol condensation, as shown below, and depends on the ready production of an anion from the phloroglucinol nucleus. Other examples of the formation of such anions are the zinc dust fissions extensively used by Boehm, and the disproportionation reactions observed by McGookin *et al.* (*loc. cit.*).

The reduction products obtained were crystalline solids, $C_{17}H_{20}O_6$, α-, m. p. 231—232°, from α-kosin and β-, m. p. 236—237°, from β-kosin, which showed a small depression of melting point on admixture. The α-compound closely resembled (XI) which has been synthesised by Boehm (*Annalen*, 1903, **329**, 283) who gives m. p. 228—229°. The α-compound gave no indophenol reaction with 2 : 6-dichloroquinonechloroimide, whereas the β-compound gave an immediate dark blue colour. The former, therefore, has no unsubstituted position *para* to a hydroxyl group, while the latter has such a free position (Gibbs, *J. Biol. Chem.*, 1927, **72**, 649). The only formula for the α-kosin product which fits this fact is (XI), and since from its origin the β-kosin product presumably has one ring identical with those in (XI) it must be either (XII; R = H, R′ = Me) or (XII; R = Me, R′ = H). Of these possibilities only the latter provides a ready explanation of its formation from protokosin (see below). The reported formation from α-kosin of only the one phloroglucinol methyl ether (VI; R = Me) (Lobeck, *loc. cit.*) is additional evidence in favour of the symmetrical formula. The compound (VI; R = Et) has $\lambda_{max.}$ 274 mμ, and (XIII) has $\lambda_{max.}$ 270·5 mμ (Mulholland, *loc. cit.*), so it was hoped that the unsymmetrical compound would show two distinguishable maxima in this region. In fact, however, the α-compound has three (271·5, 275·5, 279·3 mμ) and the β-compound two (271, 275 mμ); these absorptions are in any case quite in accord with the postulated structures and 1 : 3 : 5-trimethylphloroglucinol has two maxima (271, 274 mμ). The infra-red spectra of the kosin reduction products are very similar, but not identical. They differ from the kosins, as expected, in possessing no carbonyl band and in having a much enhanced hydroxyl band. Complete methylation of the two compounds gave the same derivative, m. p. 114—115°, which was shown to be 2 : 4 : 6 : 2′ : 4′ : 6′-hexamethoxy-3 : 3′-dimethyldiphenylmethane by synthesis, thus confirming the correctness of the skeleton assigned to the kosins. Syntheses of the kosins themselves and their reduction products are in hand.

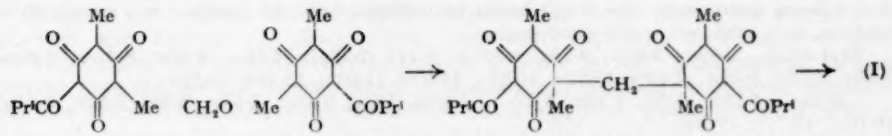
The disproportionation reactions observed by McGookin *et al.* (*loc. cit.*) raise the question whether the kosins may not in fact be symmetrical compounds produced by disproportionation of an unsymmetrical precursor formed from protokosin. However, there is no evidence of any equilibrium being set up because a third kosin has never been detected and both compounds are relatively stable in alkaline solution. The problem can only be resolved by synthesis, and in any case the general conclusions are not affected.




Formula (I) for protokosin explains its optical activity and the formation of the two kosins by migration of the angular methyl group in two possible directions, and is in accord with the absorption spectra. The unusual alkali-catalysed rearrangement is perhaps not too surprising in view of the already remarked anionoid character of the acylphloroglucinol nucleus; the driving force is presumably the tendency to form a fully aromatic system. Aromatisation by the more usual acid-catalysed type of process involving migration to carbon is impossible because of the substitution. The resemblance to the aspidin–$\psi$-aspidin change (Boehm, *Annalen*, 1903, **329**, 321) is marked, and the latter compound bears a close resemblance to the kosins from one of which it may differ, on present views, merely by the presence of *n*-butyryl instead of *iso*butyryl residues. Whether $\psi$-aspidin is in fact a single substance is not clear, but kosin was originally considered to be such before close investigation. The recorded melting point seems to vary considerably, and is lower than that of methylenebisaspidinol, with which it could be identical. Aspidin is unfortunately rather inaccessible, and we have been unable to obtain a sample of it for investigation.

The main evidence which appears at first sight to be incompatible with structure (I) is the formation of 1 : 3 : 5-trimethylphloroglucinol in the zinc dust–alkali reduction of protokosin. However, the equilibrium $R{\cdot}CH_2{\cdot}R' + H_2O \overset{OH^-}{\rightleftharpoons} R{\cdot}CH_2{\cdot}OH + R'H$ may be set up in alkaline solution (cf. Birch, *J.*, 1951, 3026; McGookin *et al.*, *loc. cit.*) or, alternatively migration of $R{\cdot}CH_2$ may occur to some extent forming $R{\cdot}CH_2{\cdot}OR''$ in a manner analogous to the migration of the methyl group which gives rise to the kosins. Either this product or $R{\cdot}CH_2{\cdot}OH$ could then react in the alkaline medium with reduction products such as dimethylphloroglucinol, and reduction of the resulting condensation product would yield trimethylphloroglucinol. It is worthy of note that in the reduction of 20 g. of protokosin, Hems and Todd (*loc. cit.*) were only able to account for a fraction of the starting material as isolated products, these being 6 g. of mixed α- and β-kosin, 1·5 g. of 1 : 3 : 5-trimethylphloroglucinol, and a small amount of *iso*butyric acid. We have, unfortunately, been unable to study this reaction further owing to the very small amount of protokosin at present available. It is pertinent that Boehm (*Annalen*, 1903, **329**, 269) found that reduction of methylenebisphloroglucinol itself gave some trimethylphloroglucinol. If the mechanism here suggested for the formation of trimethylphloroglucinol could be substantiated it would destroy the only evidence in favour of the four-membered ring (cf., *e.g.*, IV) postulated by Boehm to occur in a number of compounds from *Aspidium filix mas*, such as flavaspidic and filicic acids, and other structures which do not contain the carbon skeleton of trimethylphloroglucinol could be considered for these compounds. It is of some interest that structure (I) for protokosin could theoretically arise by the

condensation of two symmetrical units with formaldehyde, followed by a kosin-type migration of one methyl group.




## Experimental

M. p.s are uncorrected.

*Reduction of the Kosins.*—*β-Kosin.* β-Kosin (1·00 g.) in sodium hydroxide solution (20 c.c.; 10%) was stirred with 3% sodium amalgam (30 g.) during 4 hours. The aqueous solution was then separated from the liquid amalgam, which was washed with water (20 c.c.). The combined aqueous solutions were distilled rapidly until the distillate failed to react with Brady's reagent (about 10 minutes). The 2 : 4-dinitrophenylhydrazone obtained from the distillate was crystallised three times from ethanol (Found: C, 48·0; H, 4·5. Calc. for $C_{10}H_{12}O_4N_4$: C, 47·6; H, 4·8%). It had m. p. 172—174°, undepressed by an authentic specimen of the derivative of *iso*butaldehyde, m. p. 176°. The aqueous residue from the distillation was acidified, and the curdy precipitate taken up in ether (30 c.c.) and extracted successively with saturated sodium hydrogen carbonate solution, N-sodium carbonate (3 × 10 c.c.), and N-sodium hydroxide (10 c.c.). All the extracts in acidification gave some precipitate, but only that from the sodium carbonate could be crystallised. Recrystallisation of the 4 : 6 : 2′ : 4′-*tetrahydroxy*-2 : 6′-*dimethoxy*-3 : 3′-*dimethyldiphenylmethane* (?) from acetone–benzene or aqueous methanol gave colourless needles, m. p. 236—237° (decomp. after softening at *ca.* 230°) (280 mg.) (Found: C, 63·8; H, 6·4. $C_{17}H_{20}O_6$ requires C, 63·75; H, 6·25%). The absorption in the ultra-violet showed the following peaks: maxima at 275 (ε, 1750) and 271 mμ (ε, 1800), and minima at 274 (ε, 1740) and 256 mμ (ε, 1130). In a phosphate buffer at pH 9 it gave an immediate deep blue colour with 2 : 6-dichloroquinonechloroimide.

*α-Kosin.* α-Kosin (80 mg.), similarly reduced with the same proportions of reagents, gave the same 2 : 4-dinitrophenylhydrazone, m. p. 172—174°, and 4 : 6 : 4′ : 6′-*tetrahydroxy*-2 : 2′-*dimethoxy*-3 : 3′-*dimethyldiphenylmethane*, m. p. 230—231° (decomp.) (35 mg.) (Found: C, 63·8; H, 6·35. Calc. for $C_{17}H_{20}O_6$: C, 63·75; H, 6·25%). Ultra-violet absorption: maxima at 279·3 (ε, 1570), 275·5 (ε, 1730), and 271·5 mμ (ε, 1780); minima at 278·5 (ε, 1560), 273·8 (ε, 1790), and 256·5 mμ (ε, 1100). In a phosphate buffer at pH 9 it gave with 2 : 6-dichloroquinone-chloroimide only a faint pink colour which slowly became green. Methylation of this reduction product, and of that obtained above from β-kosin, gave the same compound as colourless prisms [from ether–light petroleum (b. p. 40—60°)], m. p. 114—115° (Found: C, 67·0; H, 7·3. $C_{21}H_{28}O_6$ requires C, 67·0; H, 7·4%). This was shown to be 2 : 4 : 6 : 2′ : 4′ : 6′-*hexamethoxy*-3 : 3′-*dimethyldiphenylmethane* by the following synthesis. Methylphloroglucinol trimethyl ether (700 mg.) was added to a solution of formaldehyde (40%; 0·25 c.c.) and sulphuric acid (1 c.c.) in methanol (10 c.c.), and the mixture refluxed for 10 minutes. Water (20 c.c.) was then added, the mixture extracted with ether, and the ether washed with water, dried, and evaporated. Addition of light petroleum (b. p. 40—60°) (3 c.c.) to the residue produced a crystalline precipitate which was recrystallised from ether–light petroleum (b. p. 40—60°), to give colourless prisms, m. p. 114—115°, undepressed by the compound above.

*Methylation of the Kosins.*—The kosins were difficult to methylate completely, but the alternate addition of methyl sulphate and N-sodium hydroxide on the steam-bath under nitrogen was carried out until a test-portion of the gummy product gave no colour with alcoholic ferric chloride. The alkali-insoluble gum was then passed in light petroleum (b. p. 40—60°) through a column of alumina, the pale yellow, apparently uniform, band being eluted with light petroleum (b. p. 40—60°). The 5 : 5′-*di*iso*butyryl*-2 : 4 : 6 : 2′ : 4′ : 6′-*hexamethoxy*-3 : 3′-*dimethyldiphenylmethane* was an almost colourless gum which could not be crystallised (Found: C, 67·8; H, 8·1. $C_{29}H_{40}O_8$ requires C, 67·4; H, 7·9%). The infra-red spectra are given below.

*Methylenebisbaeckeol.*—A mixture of baeckeol (400 mg.) (Hems and Todd, *J.*, 1940, 1208), paraformaldehyde (60 mg.), and a mixture of sulphuric acid (0·5 c.c.), water (0·5 c.c.), and methanol (5 c.c.) were shaken for 24 hours and then heated on the steam-bath for 30 minutes. The yellow needles which separated on cooling were recrystallised from acetone–methanol, to

give *methylenebisbaeckeol*, m. p. 177—178° (Found: C, 66·7; H, 7·0. $C_{27}H_{36}O_8$ requires C, 66·4; H, 7·3%). It gave a dark green colour with alcoholic ferric chloride.

*Infra-red Spectra.*—The spectra were determined for Nujol mulls in a Perkin-Elmer Model 21 double-beam instrument; the Nujol bands are omitted from the results. s = strong, m = medium, w = weak, s-b = side-band (λ in μ).

*Protokosin.* 2·96w, 3·23w, 6·20s, 6·45s-b, 7·11s, 7·38s-b, 7·85w, 8·15w, 8·45m, 8·67m, 8·99m, 9·12m, 9·65w, 10·01w, 10·33w, 10·63w, 10·80w, 11·02m, 13·13w, 13·77w.

α-*Kosin.* 3·13m, 6·20s, 7·10m, 7·40s-b, 7·67w, 7·87s, 8·43m, 8·63w, 9·00s, 9·35w, 9·84w, 10·10m, 10·77w, 12·3w.

β-*Kosin.* 3·10m, 6·24s, 7·13m, 7·34m, 7·63w, 7·89m, 8·45s, 9·02s, 9·34w, 9·85m, 10·05m, 10·80w, 11·2w, 12·1w.

α-*Kosin reduction product.* 3·0s, 6·11m, 6·17m, 6·60m, 7·31m, 7·66m, 7·90w, 8·16w, 8·28w, 8·80s, 9·30s, 9·90w, 11·19w, 11·55w, 12·51s, 12·85w, 13·10w, 13·90w.

β-*Kosin reduction product.* 3·0m, 6·13w, 6·20w, 6·62w, 7·34s-b, 7·7w, 7·90w, 8·18w, 8·30w, 8·82s, 9·33s, 9·90w, 12·53m.

*Flavaspidic acid.* 3·16w, 6·10s, 6·20s, 7·75m, 7·9m, 8·38s, 8·65m, 9·74w, 10·8w, 11·14w, 11·85w, 12·45w, 12·94w, 13·85w.

*Albaspidin.* 2·97w, 6·13s, 6·38s, 7·54w, 7·77m, 8·14w, 8·33m, 9·60w, 10·44w, 10·66w, 11·4w, 11·67w, 12·26w, 12·45w, 12·66w, 13·57w.

*Methylated α- and β-kosin* (β- *in brackets*). 5·87(5·89)s, 6·31(6·32)s, 6·87(6·87)s, 7·11(7·11)s, 7·24(7·24)m, 7·57(7·57)m, 7·9(7·87)w, 8·36(8·37)s, 9·03(9·03)s, 9·50(9·50)m, 9·90(9·90)s, 10·17(10·17)s-b, 10·53(10·53)w, 10·77(10·80)w, 10·94(10·96)m, 11·23(11·23)w, 11·55(11·55)w, —(11·85)w, 12·40(12·40)w.

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## 595. *Properties and Reactions of Free Alkyl Radicals in Solution. Part IV.* The Direct Reaction between Alkyl Radicals and Aliphatic Aldehydes.*

By E. F. P. HARRIS and WILLIAM A. WATERS.

The free radical $\cdot CMe_2 \cdot CO_2Me$ attacks saturated aliphatic aldehydes at 80—110° to a slight extent only. Decarbonylation of the resulting R·CO· radicals then follows as a short chain reaction:

$$R\cdot CO\cdot \longrightarrow R\cdot + CO;\ R\cdot + R\cdot CHO \longrightarrow RH + R\cdot CO\cdot$$

and small amounts of the corresponding paraffin hydrocarbons are formed. There is some indication that chain ending leads to the production of the keto-esters $R\cdot CO\cdot CMe_2\cdot CO_2Me$. Carbon tetrachloride effectively stops the decarbonylation chain by reacting with the R·CO· radicals to form acyl chlorides.

IN the vapour phase it has been established conclusively (Steacie, "Atomic and Free Radical Reactions," 1946, Reinhold Publ. Corp., N.Y., pp. 120—132, 178—200) that both the thermal and the photochemical decomposition of aldehydes are predominantly radical-chain processes:

$$X\cdot + R\cdot CHO \longrightarrow XH + R\cdot CO\cdot \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$
$$R\cdot CO\cdot \longrightarrow R\cdot + CO \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$
$$R\cdot + R\cdot CHO \longrightarrow RH + R\cdot CO\cdot \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

in which the chain-initiating radical X· may be either the radical R· resulting from a primary decomposition, $R\cdot CHO \longrightarrow R\cdot + \cdot CHO$, or atomic hydrogen produced by the breakdown of the unstable ·CHO radical (cf. equation 2). Even under favourable

* Part III, *J.*, 1952, 2240.

conditions the chain lengths for the decarbonylations of the lower aliphatic aldehydes are short and it has been concluded that both processes (2) and (3) require an appreciable activation energy.

Hitherto this reaction has been little studied in the liquid phase. In a brief note Winstein and Seubold (*J. Amer. Chem. Soc.*, 1947, **69**, 2916) have reported 90% decarbonylation of β-phenyl*iso*valeraldehyde in 5 hours at 130°, when methyl radicals derived from the thermal decomposition of di-*tert.*-butyl peroxide are used as initiators, but they observed scarcely any reaction at 80° when using benzoyl peroxide as their catalyst. Kharasch, Urry, and Kuderna (*J. Org. Chem.*, 1949, **14**, 248) found that the decarbonylation of *n*-heptanal occurs at as low a temperature as 65° as a side reaction in the acetyl peroxide-catalysed addition of this aldehyde to olefins, and was evident even at 0° in conjunction with the photochemical addition of acetaldehyde to oct-1-ene. At this low temperature it was at first doubted whether *free* R·CO· radicals are formed (Bamford and Norrish, *J.*, 1938, 1531), but the successful addition of acetaldehyde to oct-1-ene under these conditions must be regarded as conclusive evidence. From this experiment Kharasch, Urry, and Kuderna also isolated a little diacetyl, and concluded therefore that the chain-ending reaction was probably (4) :

$$2R\cdot CO\cdot \longrightarrow R\cdot CO\cdot CO\cdot R \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

They also noted that only the saturated aliphatic aldehydes lost carbon monoxide.

Since 2-carbomethoxy-2-propyl radicals, $\cdot CMe_2\cdot CO_2Me$, can conveniently be made by the thermal decomposition of dimethyl αα'-azo*iso*butyrate in homogeneous solution in the temperature range of 80—110°, and both the natures and the relative percentages of their self-interaction products have been studied accurately (Bickel and Waters, *Rec. Trav. chim.*, 1950, **69**, 312), we have used these radicals, as X· in equation (1), to initiate the liquid-phase decarbonylation of aliphatic aldehydes. In all cases the extent of reaction (1) between the $\cdot CMe_2\cdot CO_2Me$ radicals and the aldehyde was so slight that clear evidence of it could only be obtained by using high concentrations of the azo-ester and volumes of material sufficiently large to permit careful fractional distillations of the eventual reaction products. Table 1 summarises the results obtained by heating solutions of the azo-ester in aldehydes for about an hour at 80—110° so that a slow, controllable reaction occurred.

TABLE 1. *The decomposition of dimethyl αα'-azoiso*butyrate *in aliphatic aldehydes.*

| | | *n*-Heptanal | *n*-Octanal | *n*-Decanal | 3 : 5 : 5-Trimethylhexanal | 2-Ethylhexanal |
|---|---|---|---|---|---|---|
| Quantity of aldehyde ..... | g. | 34·5 | 38·5 | 46·9 | 35·6 | 48·5 |
| | mol. | 0·30 | 0·30 | 0·30 | 0·25 | 0·38 |
| Quantity of azo-ester..... | g. | 11·35 | 11·25 | 11·6 | 15·6 | 14·2 |
| | mol. | 0·049 | 0·05 | 0·05 | 0·068 | 0·062 |
| mol. fraction | | 0·16 | 0·17 | 0·17 | 0·27 | 0·16 |
| Carbon monoxide evolved | c.c. | 700 | 445 | — | 750 | — |
| | g. | 0·9 | 0·5 | 0·95 | 0·9 | 0·95 |
| as % of aldehyde ..... | | 11 | 6 | 11 | 13 | 9 |
| as % of $\cdot CMe_2\cdot CO_2Me$ radicals ..... | | 31 | 20 | 32 | 25 | 29 |
| | | *n*-Hexane | *n*-Heptane | *n*-Nonane | 2 : 4 : 4-Trimethylpentane | *n*-Heptane |
| Hydrocarbon (RH) isolated ..... | g. | 1·1 | 0·9 | 1·8 | 2·3 | 1·5 |
| Methyl *iso*butyrate isolated ..... | g. | 1·47 | 1·3 | 1·8 | 2·75 | 2·7 |
| as % of $\cdot CMe_2\cdot CO_2Me$ radicals * ..... | | 15 | 13 | 18 | 22 | 21 |

* In an inert solvent 15% of methyl *iso*butyrate is formed (Bickel and Waters, *loc. cit.*).

From each reaction the total loss of gaseous products was 60—70% more than that corresponding to the theoretical loss of nitrogen from the azo-ester, and analysis established that carbon monoxide had been liberated. The fractions of lowest boiling point were in each case the paraffins, RH, corresponding to the aldehydes R·CHO. These paraffins were characterised carefully by elementary analysis and physical constants, so as to confirm that at the temperature used there did not occur any degradation of the alkyl radicals,

$C_6H_{13}$·, etc., to olefins by the disproportionation reactions which are known to occur in the higher-temperature gas reactions.

The small amount of methyl *iso*butyrate formed in aldehyde solutions shows that most of the carbon monoxide formation must be due to a chain reaction in which the aldehyde molecules R·CHO are attacked by alkyl radicals R·.

Careful searches were made amongst the higher-boiling fractions of the various reaction mixtures for substances diagnostic of particular chain endings. Possibilities are (*a*) higher paraffins, R·R; (*b*) high-boiling ketones or 1 : 2-diketones, R·CO·R, R·CO·CO·R; (*c*) esters, $R·CMe_2·CO_2Me$; (*d*) ketonic esters, $R·CO·CMe_2·CO_2Me$. The isolation of products, however, is an extremely difficult task since the attack on the aldehyde is so slight, and consequently there always preponderate reaction products such as $MeO_2C·CMe_2·CMe_2·CO_2Me$, $MeO_2C·CMe_2·CH_2·CHMe·CO_2Me$, and $MeO_2C·CMe_2·CH_2·CMe(CO_2Me)·CMe_2·CO_2Me$ which arise from the dimerisation and disproportionation of the $·CMe_2·CO_2Me$ radicals (Bickel and Waters, *loc. cit.*). There is moreover the serious difficulty that the *n*-aldehydes easily undergo self-condensation and subsequent dehydration (to substances of crotonaldehyde type) when heated, giving high-boiling products which readily interact with all reagents that can be used for the characterisation of ketones. Nevertheless some indication of the occurrence of reaction (5), viz.:

$$R·CO + ·CMe_2·CO_2Me \longrightarrow R·CO·CMe_2·CO_2Me \quad . \quad . \quad . \quad . \quad (5)$$

has been obtained with heptanal, since on hydrolysis the high-boiling residues yielded a little *iso*butyric acid which was characterised as its *S*-benzylthiuronium salt. Compounds of type (*c*) may be present though this is not certain, and there was no evidence for the occurrence of reaction (4). Chain ending by (5) is to be expected under our experimental conditions, since it involves the two radicals of least reactivity in the whole system.

Winstein and Seubold (*loc. cit.*) also reported that with both β-phenyl*iso*valeraldehyde and *iso*valeraldehyde the reaction chain (2, 3) was inhibited when carbon tetrachloride was added to the decomposing mixture, owing to the intervention of reactions (6) and (7):

$$R·CO· + CCl_4 \longrightarrow R·COCl + ·CCl_3 \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$
$$R·CHO + ·CCl_3 \longrightarrow R·CO· + CHCl_3 \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

We have found that this reaction is general. As Table 2 shows, very little carbon monoxide indeed is evolved from decompositions of dimethyl αα′-azo*iso*butyrate in dry aldehyde–carbon tetrachloride mixtures under nitrogen. The products of these reactions were fractionated and the portions of both lower and higher boiling point were then treated with water. Significant amounts of ionisable chlorine were found to be associated with the fractions of boiling point 130° or over, so that acyl chlorides are undoubtedly formed. When air was not excluded, some carbonyl chloride was formed: this is indicative of the presence of $·CCl_3$ radicals, and did not occur in the absence of the aldehydes (cf. Part III). Attempts to isolate the small amounts (1—2 g.) of the acyl chlorides failed because of the closeness of their boiling points to those of the corresponding aldehydes. Though the expected carboxylic acids were formed by hydrolysis it may be argued that these could have arisen by autoxidation of the aldehyde during the necessary manipulations.

TABLE 2. *Decompositions of dimethyl αα′-azoisobutyrate in aldehyde–carbon tetrachloride mixtures.*

Each mixture contained 0·1 mole of aldehyde, 0·2 mole of carbon tetrachloride, and 0·016 mole (3·85 g.) of the azo-ester.

| Aldehyde | Total gas evolved, c.c. (N.T.P.) | CO, c.c. | Acyl chloride (by titration): mol. $\times 10^{-3}$ (= % of R·CHO) | Acyl chloride (by titration): as % of $·CMe_2·CO_2Me$ radicals |
|---|---|---|---|---|
| None | 375 | — | — | — |
| Benzaldehyde | 372 | — | 3 | 9 |
| *n*-Heptanal | 370 | — | 12 | 36 |
| *n*-Octanal | 385 | 10 | 6 | 19 |
| *n*-Decanal | 390 | 15 | 9·5 | 31 |
| 3 : 5 : 5-Trimethylhexanal | 396 | 21 | 17· | 54 |
| 2-Ethylhexanal | 454 | 79 | 14 | 43 |

This series of experiments showed that some reaction does occur between $\cdot CMe_2 \cdot CO_2Me$ radicals and benzaldehyde to give benzoyl radicals and thence benzoyl chloride, though the direct reaction between $\cdot CMe_2 \cdot CO_2Me$ radicals and carbon tetrachloride is insignificant. Thus, as Kharasch, Urry, and Kuderna (*loc. cit.*) have concluded, the absence of carbon monoxide formation from benzaldehyde is due to the resonance stability of the benzoyl radical, and not to any great difference in the ease of homolysis of the C–H bonds of aromatic and aliphatic aldehydes.

Tables 1 and 2 indicate that there are significant differences in reactivity between the *n*-aldehydes and their branched-chain analogues, but since, during the heating, the *n*-aldehydes may be undergoing the concurrent reaction, $2R \cdot CH_2 \cdot CHO \longrightarrow R \cdot CH_2 \cdot CH{=}CR \cdot CHO + H_2O$, we do not think that it is justifiable to suggest possible relations between chemical structure and reactivity.

## Experimental

*Decomposition of Dimethyl αα′-Azoisobutyrate.*—(1) *In* n-*heptanal.* The azo-ester (11·35 g., 0·049 mole), dissolved under nitrogen in *n*-heptanal (34·6 g., 0·303 mole) in a flask connected through a reflux condenser and a cold trap to a large gas burette, was heated gently to about 90° so that gas evolution occurred steadily at as low a temperature as possible. After 1½ hours, when reaction had ceased, the total gas evolution amounted to 1800 c.c. (corr. to N.T.P.). This corresponded almost exactly to the observed loss of weight (2·23 g.) of total products. Examination in a mass spectrometer showed that this gas consisted almost entirely of nitrogen and carbon monoxide, and that hydrogen and simple gaseous hydrocarbons were absent.* The liquid products were fractionated under dry nitrogen, giving (i) 4·1 g., b. p. 60—110°/750 mm., (ii) 25·2 g., b. p. 140—152°/750 mm., (iii) 3·9 g., b. p. 50—95°/11 mm., and (iv) 6·1 g., b. p. 99—165°/11 mm. Fraction (i) was refluxed for 2 hours with methanolic potassium hydroxide. The solution was then distilled, from a steam-bath, and the distillate was diluted with water. Two layers separated. The upper of these was washed with water, dried (KOH), and distilled. It gave 1·1 g. of *n*-hexane, b. p. 68·5°, $n_D^{16}$ 1·3782 (corr. to $n_D^{20}$ 1·3761), $d_{16}^{16}$ 0·668 (Found: C, 83·7; H, 16·6. Calc. for $C_6H_{14}$: C, 83·7; H, 16·3%). *n*-Hexane has b. p. 69·0°, $n_D^{20}$ 1·3754, $d_{16}^{20}$ 0·661.

The residue from this hydrolysis was dissolved in water, aldehydic condensation products were removed by extraction with ether, and after acidification the aqueous solution was again extracted with ether. It yielded 1·27 g. of *iso*butyric acid (equiv. to 1·47 g. of methyl *iso*butyrate) which was identified by conversion into its *S*-benzylthiuronium salt, m. p. 144°.

Fractions (ii) and (iii) consisted of unchanged heptanal. Fraction (iv) gave an orange-red 2 : 4-*dinitrophenylhydrazone*, m. p. 129·5°, which had an absorption maximum at 3850 Å (Found: C, 61·4; H, 7·4; N, 14·5. $C_{20}H_{30}O_4N_4$ requires C, 61·5; H, 7·7; N, 14·4%). By direct comparison this was shown to be identical with the 2 : 4-dinitrophenylhydrazone of the self-condensation and dehydration product of *n*-heptanal, *i.e.*, $C_6H_{13} \cdot CH{=}C(C_6H_{11}) \cdot CHO$ which, it was then found, is formed when *n*-heptanal is refluxed for an hour under nitrogen.

Fraction (iv) was refractionated under nitrogen, giving (*a*) 2·4 g., b. p. 120—130°/11 mm., and (*b*) 1·65 g., b. p. 150—152°/12 mm. Portion (*a*) was refluxed for 4 hours with methanolic potassium hydroxide. The solvent and unsaponified products were removed and the aqueous residue was acidified and distilled in steam. Volatile acids (0·9 g.) were collected in ether and, after removal of the ether, were kept at 0° for some days, whereupon part (0·6 g.) solidified. The liquid remainder smelt of *iso*butyric acid and yielded its *S*-benzylthiuronium salt of m. p. and mixed m. p. 144°. The solid acid, after crystallisation from light petroleum, proved to be tetramethylsuccinic acid. A further 0·6 g. of this substance was also isolated from the residue from the steam-distillation. Alkaline hydrolysis of (*b*) also yielded a trace of *iso*butyric acid.

(2) *In* n-*octanal.* Decomposition of the azo-ester (1·25 g.) in *n*-octanal (38·5 g.) was carried out similarly and gave 1543 c.c. of gas (N.T.P.), the loss of weight being 1·9 g. On distillation there were obtained (i) 4·1 g., b. p. 80—100°/752 mm., (ii) 25·5 g., b. p. 163—168°/752 mm., (iii) 3·1 g., b. p. 60—90°/9 mm., and (iv) 13 g., b. p. 90—200°/9 mm. Fraction (i) was dissolved in ether, extracted with aqueous sodium hydrogen sulphite to remove aldehydes, and then refluxed with methanolic potassium hydroxide for 2 hours. By following the procedure given above there was obtained 0·9 g. of *n*-heptane, b. p. 96°, $n_D^{19}$ 1·3893, $d_{17}^{17}$ 0·682 (Found: C, 83·7;

* We thank Dr. Danby of the Physical Chemistry Laboratory, Oxford, for this report.

H, 16·1. Calc. for $C_7H_{16}$: C, 84·0; H, 16·0%). *n*-Heptane has b. p. 98·4°, $n_D^{20}$ 1·3876, $d_{20}^{20}$ 0·684. The alkaline residue after acidification gave 1·15 g. of *iso*butyric acid, b. p. 150°, corresponding to 1·3 g. of methyl ester. Fractions (ii) and (iii) consisted of *n*-octanal, whilst (iv) contained 2-*n*-hexyldec-2-enal, which gave an orange-red 2 : 4-*dinitrophenylhydrazone* of m. p. and mixed m. p. 100° (Found: C, 63·9; H, 8·3; N, 13·1. $C_{22}H_{34}O_4N_4$ requires C, 63·2; H, 8·1; N, 13·4%). Only 0·1 g. of volatile acid, b. p. 140—150°, was isolated by alkaline hydrolysis of this fraction, besides 1·9 g. of tetramethylsuccinic acid.

(3) *In* n-*decanal*. The decomposition of the azo-ester (11·6 g.) in *n*-decanal (46·9 g.) gave 2·37 g. of gaseous products. There were isolated (i) 2·4 g., b. p. 60—65°/120 mm., (ii) 43·7 g., b. p. 65—95°/11 mm., (iii) 3·8 g., b. p. 95—175°/11 mm., and (iv) 3·6 g., b. p. 175—190°/11 mm. Fraction (i) after alkaline hydrolysis yielded *iso*butyric acid, b. p. 150°, equivalent to 1·8 g. of methyl ester. Fraction (ii) gave 2·8 g., b. p. 55—80°/11 mm., which after treatment with bisulphite, etc., eventually yielded 1·8 g. of *n*-nonane, b. p. 148—150°, $n_D^{35}$ 1·3980 (corr. to $n_D^{20}$ 1·4055), $d_{23}^{23}$ 0·7158 (Found: C, 83·3; H, 15·1. Calc. for $C_9H_{20}$: C, 84·4; H, 15·6%). *n*-Nonane has b. p. 150·8°, $n_D^{20}$ 1·4055, $d_{20}^{20}$ 0·7177.

The higher-boiling portion of fraction (ii), and fraction (iii), comprised unchanged aldehyde, whilst (iv) contained 2-*n*-octyldodec-2-enal, from which was prepared a 2 : 4-*dinitrophenylhydrazone*, m. p. 84° (Found: C, 65·6; H, 8·6; N, 11·7. $C_{26}H_{42}O_4N_4$ requires C, 65·6; H, 8·9; N, 11·8%).

(4) *In* 2-*ethylhexanal*. The decomposition of the azo-ester (14·2 g.) in 2-ethylhexanal (48·5 g.) gave 2·7 g. of gaseous products. Fractionation of the liquid gave (i) 7·6 g., b. p. 65—115°/760 mm., (ii) 36·3 g., b. p. 120—160°/760 mm., (iii) 2·1 g., b. p. 40—70°/10 mm., and (iv) 7·9 g., b. p. 95—153°/8 mm. After removal of aldehyde and subsequent alkaline hydrolysis fraction (i) gave 1·8 g. of *n*-heptane, b. p. 97°, $n_D^{14}$ 1·3910 (corr. to $n_D^{20}$ 1·3880), $d_{18}^{18}$ 0·687 (Found: C, 83·7; H, 16·1%). From the alkaline hydrolysis residue there were obtained 2·3 g. of *iso*butyric acid, corresponding to 2·7 g. of the methyl ester. Fractions (ii) and (iii) consisted of unchanged aldehyde, whilst (iv) contained dimethyl tetramethylsuccinate and similar products. Alkaline hydrolysis of the fractions of high boiling point yielded only a trace of *iso*butyric acid.

(5) *In* 3 : 5 : 5-*trimethylhexanal*. The decomposition of the azo-ester (15·6 g.) in 3 : 5 : 5-trimethylhexanal gave 2270 c.c. of gas (at N.T.P.); the loss of weight as gaseous products was 2·9 g. Distillation yielded (i) 5·4 g., b. p. 60—95°/759 mm., (ii) 29·0 g., b. p. 135—160°/759 mm., (iii) 6·8 g., b. p. 95—140°/12 mm., and (iv) 4·5 g., b. p. 155—195°/12 mm. Fraction (i), after alkaline hydrolysis for 4 hours, gave 2·3 g. of 2 : 4 : 4-trimethylpentane, b. p. 98—99°, $n_D^{15\cdot5}$ 1·3943, $d_{18}^{18}$ 0·694 (Found: C, 84·5; H, 15·4. Calc. for $C_8H_{18}$: C, 84·2; H, 15·8%). 2 : 4 : 4-Trimethylpentane has b. p. 99·2°, $n_D^{20}$ 1·3914, $d_{20}^{20}$ 0·692; an authentic specimen was found to have $n_D^{15\cdot5}$ 1·3949. From the alkali there were separated 2·4 g. of *iso*butyric acid, b. p. 150°, corresponding to 2·75 g. of methyl ester. Fraction (ii) consisted of unchanged aldehyde, whilst (iii) and (iv) contained dimethyl tetramethylsuccinate and similar products. The non-volatile residue yielded a trace of *iso*butyric acid after alkaline hydrolysis. No unsaturated aldehyde was formed under these conditions.

*Decompositions in the Presence of Carbon Tetrachloride.*—The decompositions of dimethyl αα′-azo*iso*butyrate in aldehyde–carbon tetrachloride mixtures, summarised in Table 2, were carried out under similar conditions: in each case 3·85 g. (0·0167 mole) of the azo-ester, 0·10 mole of the aldehyde, and 0·20 mole of carbon tetrachloride were used. The following reaction with *n*-heptanal is typical.

The mixture was heated gently till gas evolution set in, and it was finally refluxed for 4 hours to ensure complete reaction. Air had previously been displaced from the apparatus by dry nitrogen. The gas evolved was equivalent to 370 c.c. at N.T.P., whilst the calculated volume of nitrogen liberated from the azo-ester is 375 c.c. The liquid products were fractionally distilled in dry nitrogen. The carbon tetrachloride portion was acid, and washing with water gave a solution containing, by Volhard titration, 0·012 g. of chloride anion.

The fraction of higher boiling point was washed with 2N-ammonia; this aqueous layer was then made up to a standard volume and its chloride content was determined by Volhard titrations of aliquot parts. There had been liberated 0·012 mole of chloride anions, equivalent to formation of 1·73 g. of *n*-heptanoyl chloride. Characterisation both as amide and as anilide was attempted, but in each case the condensation of the free aldehyde with the base vitiated success.

When these decompositions were carried out in air, acrid fumes were evolved both during the decomposition of the azo-ester and during the distillation of the carbon tetrachloride later. By reaction of the vapours with aniline it was shown that carbonyl chloride was present in

appreciable amount. This was not noticeable when the azo-ester was boiled in carbon tetrachloride in the absence of an aldehyde.

We thank Imperial Chemical Industries Limited, Billingham, for gifts of pure specimens of aldehydes and of 2 : 4 : 4-trimethylpentane. Microanalyses were by Drs. Weiler and Strauss.

THE DYSON PERRINS LABORATORY, OXFORD. [*Received, April 8th,* 1952.]

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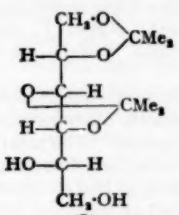
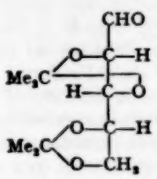
## 596. *The* 2 : 3-4 : 5-*Diisopropylidene Derivatives of* aldehydo-L-*Xylose and* L-*Xylitol.*

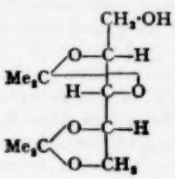
By E. J. BOURNE, G. P. MCSWEENEY, and L. F. WIGGINS.

2 : 3-4 : 5-Di*iso*propylidene *aldehydo*-L-xylose, prepared from 1 : 2-3 : 4-di*iso*propylidene L-iditol by oxidation with periodate, has been characterised and some of its crystalline derivatives are described. It has been converted into 2 : 3-4 : 5-di*iso*propylidene L-xylitol by hydrogenation.

IN a previous paper (Bourne, McSweeney, and Wiggins, *J.*, 1952, 2542), part of the evidence presented in support of the conclusion that di*iso*propylidene L-iditol (I) carries the acetone residues in the 1 : 2- and 3 : 4-positions was that the compound consumed 1 mol. of periodate with the concomitant production of formaldehyde, and it was noted that the major product of the oxidation should have been 2 : 3-4 : 5-di*iso*propylidene *aldehydo*-L-xylose (II), a compound which has been obtained hitherto only in admixture with 2 : 3-4 : 5-di*iso*propylidene *aldehydo*-D-arabinose (Bourne, McSweeney, Stacey, and Wiggins, *J.*, 1952, 1408). We have now repeated the oxidation on a larger scale and have, in fact, isolated a syrup, analysis of which was correct for a di*iso*propylidene pentose. As confirmation that the pentose moiety was L-xylose, the sugar produced on hydrolysis of this syrup yielded the known β-tetra-acetyl L-xylose with acetic anhydride–sodium acetate, and, when submitted to filter-paper chromatography, gave one spot, having an $R_F$ value identical with that of an authentic specimen of xylose, used as a standard on the same chromatogram.


(I)


(II)


(III)

2 : 3-4 : 5-Di*iso*propylidene *aldehydo*-L-xylose exhibited the usual aldehydic properties inasmuch as it gave a semicarbazone, an oxime, and a bisdimedone derivative, all crystalline. The bisdimedone compound, like that of the isomeric 2 : 3-4 : 5-di*iso*propylidene *aldehydo*-D-arabinose (*loc. cit.*), was insoluble in water, a fact which explained why, in our previous work (*J.*, 1952, 2542), the formaldehyde liberated during periodate oxidation of 1 : 2-3 : 4-di*iso*propylidene L-iditol could be characterised as its water-insoluble dimedone derivative only after it had been purified by distillation in steam.

Hydrogenation of 2 : 3-4 : 5-di*iso*propylidene *aldehydo*-L-xylose in ethyl alcohol in the presence of Adams's platinum oxide afforded a syrupy non-reducing di*iso*propylidene pentitol, which gave a crystalline toluene-*p*-sulphonate. In view of the route by which it had been prepared, there can be little doubt that this syrup was 2 : 3-4 : 5-di*iso*propylidene L-xylitol (III, synonymous with 1 : 2-3 : 4-di*iso*propylidene D-xylitol); it was probably the L-isomer of 2 : 3-4 : 5-di*iso*propylidene DL-xylitol which results from the treatment of xylitol with acetone, anhydrous copper sulphate, and concentrated sulphuric acid (cf.

Tipson and Cretcher, *J. Org. Chem.*, 1943, **8**, 95; Hann, Ness, and Hudson, *J. Amer. Chem. Soc.*, 1944, **66**, 73).

## Experimental

**1 : 2-3 : 4-5 : 6-Tri*iso*propylidene L-iditol**, prepared as described by Bourne, McSweeney, and Wiggins (*loc. cit.*), had m. p. 57—58° alone or on admixture with an authentic specimen, $[\alpha]_D^{16}$ +5·8° (*c*, 5·5 in chloroform) (Found: C, 59·5; H, 8·6. Calc. for $C_{15}H_{26}O_6$: C, 59·6; H, 8·7%).

**1 : 2-3 : 4-Di*iso*propylidene L-iditol** (*loc. cit.*) had b. p. 136—140° (bath-temp.)/0·005 mm., $[\alpha]_D^{19}$ +23·8° (*c*, 2·9 in ethyl alcohol) (Found: C, 54·8; H, 8·5. Calc. for $C_{12}H_{22}O_6$: C, 54·9; H, 8·5%).

**2 : 3-4 : 5-*Di*iso*propylidene* aldehydo-L-*Xylose***.—To an aqueous solution (20 c.c.) of 1 : 2-3 : 4-di*iso*propylidene L-iditol (6·50 g.) a solution (20 c.c.) of sodium metaperiodate (6·60 g.) was added slowly at 0°. After 24 hours, the solution was saturated with sodium chloride and extracted exhaustively with chloroform. The extracts were dried ($MgSO_4$), filtered, and evaporated at 40° to a syrup (5·0 g.), which was fractionally distilled. The main fraction, **2 : 3-4 : 5-*di*iso*propylidene* aldehydo-L-*xylose***, was a colourless mobile syrup (3·24 g.), b. p. 92—100° (bath-temp.)/0·04 mm., $n_D^{16}$ 1·4545, $[\alpha]_D^{19}$ +25·6° (*c*, 3·2 in ethyl alcohol), $[\alpha]_D^{18}$ +25·2° (*c*, 3·9 in methyl alcohol) (Found: C, 57·0; H, 8·0. $C_{11}H_{18}O_5$ requires C, 57·4; H, 7·9%). The syrup became viscous when kept at room temperature for a day. The ***semicarbazone*** had m. p. 175—176°, $[\alpha]_D^{18}$ +29·4° (*c*, 2·7 in chloroform) (Found: C, 50·3; H, 7·6; N, 14·3. $C_{12}H_{21}O_5N_3$ requires C, 50·2; H, 7·4; N, 14·6%). The ***oxime*** had m. p. 90—91°, $[\alpha]_D^{19}$ +19·1° (*c*, 2·5 in ethyl alcohol) (Found: C, 53·7; H, 7·8; N, 5·7. $C_{11}H_{19}O_5N$ requires C, 53·9; H, 7·8; N, 5·7%). The ***bisdimedone*** derivative had m. p. 111—112°, $[\alpha]_D^{16}$ −26·6° (*c*, 3·8 in chloroform) (Found: C, 65·9; H, 8·3. $C_{27}H_{40}O_8$ requires C, 65·8; H, 8·2%).

*Hydrolysis of* **2 : 3-4 : 5-*Di*iso*propylidene* aldehydo-L-*Xylose***.—The xylose derivative (1·00 g.) was treated with N-sulphuric acid at 100° for 3 hours; the solution was neutralised with barium carbonate and filtered, the filtrate being evaporated to a syrup (0·60 g.). Paper-chromatographic analysis of a portion of the syrup, with the upper phase of a mixture of butanol (40%), ethanol (10%), ammonia (1%), and water (49%) as the solvent, and a butanol solution of aniline hydrogen phthalate as the spray (cf. Partridge, *Nature*, 1949, **164**, 443), revealed one component; this had an $R_F$ value identical with that of xylose, used as a standard on the same chromatogram.

The remainder of the syrup was treated with acetic anhydride (3 c.c.) and fused sodium acetate (0·8 g.) at 100° for 1 hour. The precipitate obtained when the reaction mixture was poured into water was recrystallised from aqueous ethyl alcohol, to give tetra-acetyl β-L-xylose (0·40 g.), m. p. and mixed m. p. 125°, $[\alpha]_D^{22}$ +25·8° (*c*, 2·0 in chloroform). Appel (*J.*, 1935, 425) gave m. p. 127—128·5°, $[\alpha]_D^{15}$ +25·7° (in chloroform) for this compound; Vargha (*Ber.*, 1935, **68**, 18) recorded m. p. 126°, $[\alpha]_D^{20}$ +25·7° (in chloroform).

**2 : 3-4 : 5-*Di*iso*propylidene* L-*Xylitol***.—A solution of **2 : 3-4 : 5-di*iso*propylidene *aldehydo*-L-xylose** (2·00 g.) in ethyl alcohol (20 c.c.) was shaken with Adams's platinum oxide catalyst (0·07 g.) in the presence of hydrogen under slight pressure until absorption ceased. The mixture was filtered and the filtrate was evaporated at 12 mm. to a syrup, which was then fractionally distilled. The main fraction, **2 : 3-4 : 5-*di*iso*propylidene* L-*xylitol*** (1·60 g.), had b. p. 96—98° (bath-temp.)/0·03 mm., $n_D^{21}$ 1·4570, $[\alpha]_D^{18}$ +12·5° (*c*, 3·6 in ethyl alcohol) (Found: C, 56·8; H, 8·8. $C_{11}H_{20}O_5$ requires C, 56·9; H, 8·7%). **2 : 3-4 : 5-Di*iso*propylidene DL-xylitol**, prepared directly from xylitol and acetone, has m. p. 33—34·5° (Tipson and Cretcher, *loc. cit.*; Hann, Ness, and Hudson, *loc. cit.*), and can be crystallised only with difficulty in the absence of seed crystals.

**2 : 3-4 : 5-*Di*iso*propylidene* 1-*toluene*-p-*sulphonyl* L-*xylitol***, prepared by toluene-*p*-sulphonyl chloride in dry pyridine at 25° (20 hours), crystallised from aqueous ethyl alcohol as needles, m. p. 65—66°, $[\alpha]_D^{20}$ +16·3° (*c*, 4·4 in ethyl alcohol) (Found: C, 56·0; H, 6·8; S, 8·1. $C_{18}H_{26}O_7S$ requires C, 55·9; H, 6·8; S, 8·3%). Like **2 : 3-4 : 5-di*iso*propylidene 1-toluene-*p*-sulphonyl DL-xylitol**, the L-isomer decomposed spontaneously in a few days at room temperature. Recorded values of the m. p. of the DL-isomer are 70—71° (Tipson and Cretcher, *loc. cit.*) and 77—78° (Hann, Ness, and Hudson, *loc. cit.*).

The authors are indebted to Professor M. Stacey, F.R.S., for his interest, to the Tootal, Broadhurst, Lee Co. Ltd. for a grant, and to Miss E. Fowler for carrying out the microanalyses.

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## 597. *Studies on Bond Type in Certain Cobalt Complexes. Part I. Exchange Reactions.*

By B. West.

The exchange reactions with cobaltous ions in pyridine or water solution of nine cobaltous and three cobaltic complexes of the bidentate, chelate type have been examined, radioactive cobalt being used. The cobaltous complexes with salicylaldehyde, salicylideneaniline, salicylidene-*m*-toluidine, salicylidene-*o*-anisidine, acetylacetone, guaiacol, and salicylaldoxime have been shown to undergo very rapid exchange. Cobaltous bis-(4 : 4′-dicarbethoxy-3 : 3′ : 5 : 5′-tetramethyldipyrromethene) exchanges slowly, with $t_{\frac{1}{2}} \simeq 51{\cdot}5$ hours. Cobaltous phthalocyanine does not show exchange nor does tris-ethylenediaminecobalt trichloride. Tris-α-nitroso-β-naphthol- and tris-acetylacetone-cobalt show evidence for a very slow exchange. The results have been interpreted in terms of bond type between the cobalt atom and its ligands, slow exchange being taken as evidence for partial ionic character, and fast exchange for strong ionic character.

The investigation of the exchange of metal ions between a complex molecule or ion containing the metal and free metal ions in a suitable solvent makes it possible to obtain some evidence as to the types of bonds present in the complex. If the bonding is predominantly ionic, exchange is expected to occur rapidly, probably too fast to be measured. As the bond type changes towards predominantly covalent linking, the rate of exchange is expected to decrease. Where exchange is observed to proceed at a measurable rate, the "mechanism" of exchange must be first established before unequivocal statements as to bond type can be made, *i.e.*, the mechanism must be shown to be that due to (*a*) an ionisation and recombination by the complex, or (*b*) a collision followed by a replacement of the bound metal by the colliding metal ion, or (*c*) the racemization of an optically active complex. Duffield and Calvin (*J. Amer. Chem. Soc.*, 1946, **68**, 557) have shown that a distinction can be drawn between the first and the second mechanism on kinetic grounds. An ionisation–recombination mechanism requires the half-time for the reaction to be independent of the concentrations of the exchanging species, but for a collision mechanism to be operative, the half-time is inversely proportional to concentration; in both cases, concentrations are assumed equal to each other. Chemical considerations should, in general, cover the final case.

At the present stage of knowledge of exchange reactions with chelate-type complexes it seems possible to obtain qualitative evidence as to the strength of bonding by observing whether an exchange is fast, slow, or intermediate in rate without being certain of the exact mechanism of exchange. If strong covalencies exist in a complex, then exchange will be expected to be slow whether it proceeds by an ionisation or a collision mechanism. In the first case, ionisation would be very slight for such a compound. For weak covalent bonds or ionic bonds, rapid exchange could occur by both mechanisms. This procedure has been adopted in the present paper.

### Experimental.

*Materials.*—The pyridine used was of "AnalaR" quality, dried by refluxing over barium oxide followed by fractional distillation whenever required. The fraction of b. p. 115·3° was collected for use.

Chloroform was washed several times with water, dried ($CaCl_2$), and distilled over fresh calcium chloride. Batches were stabilised by the addition of 2% of ethyl alcohol but were never used without fresh treatment if kept longer than two weeks. No trace of carbonyl chloride or hydrogen chloride was detected in any samples used.

Distilled water was used whenever required.

*Radioactive Cobalt.*—This was supplied by the Isotopes Division of the American A.E.C. as the isotope $^{60}Co$ in a solution of carrier cobalt sulphate containing small amounts of sulphuric acid and potassium sulphate. It was purified by a double precipitation as sulphide, acetate buffer and ammonium sulphide being used. The product was dissolved in dilute sulphuric acid, and the

solution boiled to remove hydrogen sulphide, etc., and evaporated to dryness. The product was then dissolved in water, and small volumes were removed when required for the preparation of solutions. Cobalt sulphate solutions containing the isotope were prepared by weighing out the required amount of the heptahydrate, dissolving it in water, and adding a quantity of the radioactive stock solution. The whole solution was then diluted to the appropriate volume.

Cobalt acetate solutions were prepared by weighing out the quantity of tetrahydrate salt required, adding radioactive stock solution together with a few drops of glacial acetic acid, and evaporating the mixture to dryness with an infra-red lamp. The dry residue was dissolved in pyridine and diluted to the appropriate volume.

*Compounds.*—The salicylideneaniline type complexes were prepared by using the method developed by Bailes and Calvin (*J. Amer. Chem. Soc.*, 1947, **69**, 1886) for preparing cobaltous compounds out of contact with air. The Schiff's base was first prepared by warming equivalent amounts of salicylaldehyde and amine in ethyl alcohol, evacuating, then adding the requisite amount of cobalt acetate dissolved in warm water, shaking, and re-evacuating. The following complexes, precipitated as red crystals, were filtered off and recrystallised several times from absolute alcohol. Solutions were prepared by dissolving weighed amounts in pyridine and making up to the required volume. Bis(salicylideneaniline)cobalt(II) [Found: C, 69·2; H, 4·5. Calc. for $Co(C_{13}H_{10}ON)_2$: C, 69·2; H, 4·5%]; bis(salicylidene-*m*-toluidine)cobalt(II) [Found: C, 69·8; H, 5·04. Calc. for $Co(C_{14}H_{12}ON)_2$: C, 70·1; H, 5·05%]; bis(salicylidene-*o*-anisidine)cobalt(II) [Found: 65·9; H, 4·8. Calc. for $Co(C_{14}H_{12}O_2N)_2$: C, 65·75; H, 4·7%]

Bis(guaiacol)cobalt(II) was prepared by Mellor and Craig's method (*J. Proc. Roy. Soc. N.S.W.*, 1940, **74**, 495) [Found: C, 55·4; H, 5·6. Calc. for $Co(C_7H_7O_2)_2$: C, 56·1; H, 4·6%].

Bis(acetylacetone)cobalt(II) [Found: C, 40·9; H, 6·6. Calc. for $Co(C_5H_7O_2)_2,2H_2O$: C, 40·7; H, 6·8%] and bis(salicylaldehyde)cobalt(II) [Found: C, 48·6; H, 4·5. Calc for $Co(C_7H_5O_2)_2,2·5H_2O$: C, 48·6; H, 4·1%] were prepared as hydrates by adding the calculated amount of cobaltous chloride hexahydrate dissolved in water to an alcohol solution of the base, and crystallised from aqueous alcohol.

Bis-(4:4′-dicarbethoxy-3:3′:5:5′-tetramethyldipyrromethene)cobalt(II) was prepared by a method analogous to that used by Mellor and Lockwood (*ibid.*, 1940, **74**, 141) for the nickel derivative, and recrystallised from absolute alcohol [Found: C, 61·5; H, 6·4. Calc. for $Co(C_{19}H_{23}O_4N_2)_2$: C, 61·2; H, 6·22%]. Bis(salicylaldoxime)cobalt(II), prepared by the addition of an aqueous solution of the chloride hexahydrate to an alcoholic solution of the base, formed a brown amorphous product which could not be recrystallised [Found: C, 49·4, 49·4; H, 3·72. Calc. for $Co(C_7H_6O_2N)_2$: C, 50·8; H, 3·65%]. This sample was used in the first exchange experiments with this compound. However, in view of later work (see Discussion, p. 3119) it seems probable that the complex prepared in this manner has taken up oxygen, forming a mixed product which could contain an oxygenated derivative together with some bis(salicylaldoxime)cobalt.

Samples of cobaltous phthalocyanine and of the ammonium salt of the tetrasulphonic acid derivative were kindly supplied by Dr. M. Rogers through the courtesy of Imperial Chemical Industries Limited

The tris(ethylenediamine)cobalt(III) chloride complex was prepared as a hexahydrate (*Inorg. Synth.*, **2**, 221) (Found: C, 18·0; H, 8·1; N, 21·5; Co, 15·0. Calc. for $C_6H_{24}N_6Cl_3Co,6H_2O$: C, 18·3; H, 9·2; N, 21·4; Co, 15·0%). Tris-(1-nitroso-2-naphthol)cobalt(III) was prepared as recommended by Vogel ("Text Book of Quantitative Analysis," Longmans, 1948, p. 547) [Found: C, 62·6; H, 3·5. Calc. for $Co(C_{10}H_6O_2N)_3$: C, 62·6; H, 3·15%]. Tris(acetylacetone)-cobalt(III) was prepared by Urbain and Debierne's method (*Compt. rend.*, 1899, **129**, 302) and recrystallised from absolute alcohol. The green, crystalline product had m. p. 196·0° (uncorr.); Urbain and Debierne give 241° [Found: C, 50·2; H, 5·9. Calc. for $Co(C_5H_7O_2)_3$: C, 50·6; H, 5·9%].

*Method.*—Several methods were used to follow the exchange. The usual one was to add 5 c.c. of a pyridine solution of the complex compound (0·017M) in a 150-c.c. separating funnel held in a water-thermostat at the required temperature. When the solution reached temperature equilibrium, 5 c.c. of radioactive cobalt acetate (0·017M) were added from a storage vessel kept at bath-temperature. After the required time, a mixture of 14 c.c. of chloroform and 14 c.c. of water was added, and the mixture shaken. Each layer was then re-extracted once with 14 c.c. of the appropriate solvent. The extracts were combined and made up to 50 c.c. with ethyl alcohol in volumetric flasks. Aliquots were then counted in either 10-c.c. or 20-c.c. glass-skirted Geiger counters. For water-soluble complexes, reaction mixtures were prepared as above but separation was effected by the addition of 6 g. of ammonium thiocyanate and 10 c.c.

of an amyl alcohol–ether mixture (4 : 1). Each layer was re-extracted once more with appropriate solvent, the extracts made up to 50 c.c., and aliquots counted.

Bis(guaiacol)cobalt being almost insoluble in all conventional solvents, a weighed sample was shaken for various lengths of time with 5 c.c. of aqueous radioactive cobalt sulphate solution (0·017M) plus 5 c.c. of ethyl alcohol. The weight of complex used contained an equivalent amount of cobalt. The solution was filtered through a sintered-glass funnel, the filtrate made up to 50 c.c., and the residue dissolved in dilute hydrochloric acid (1 : 3) and also made up to 50 c.c. An aliquot of each solution was then counted.

In the second series of experiments with bis(salicylaldoxime)cobalt, the complex was preformed in pyridine solution by dissolving equivalent weighed amounts of cobalt acetate and reagent in 40 c.c. of solvent to give a solution 0·0085M. The solution was prepared under nitrogen and after complete solution of the materials had occurred a further 60 minutes was allowed for completion of chelation. Then 20 c.c. of a 0·017M-pyridine solution of radioactive cobalt acetate were added, and aliquots removed under nitrogen pressure at suitable intervals, and shaken with chloroform and water. The colour of the solution of the complex thus formed was light yellow at this concentration, but on exposure to air a deep brown solution resulted similar to that obtained with the amorphous product used in the first instance.

In each case where a solvent-extraction method was used a standard extraction using 5 c.c. of the radioactive solution plus 5 c.c. of pyridine (or water) was carried out in order to check distribution. This effect has been allowed for in each case. The extraction of most complexes showed that some chelate was held in the aqueous layer even after double extractions. For two cases, bis(salicylideneaniline)cobalt and bis(salicylidene-*m*-toluidine)cobalt, the total cobalt in each layer after extraction was determined in inactive solutions by precipitation of the sulphide and ignition to $Co_3O_4$. This allows corrections to be made for uneven distribution of activity. In other cases, constancy of activity in the chloroform solution over long time intervals when almost equal to that expected for complete exchange has been taken to imply that complete exchange has occurred. Since the object of this research was not primarily accurate kinetic measurements, but rather the surveying of groups of compounds, the above procedure has proved sufficient for the purpose. Measurements of activity were carried out with an Austronic (Australian) scale of 100 units and liquid-type Alltool counters. Corrections for background were applied in all cases, but no corrections were needed for radioactive decay ($^{60}Co$ half-life = 5·3 years; Livingwood and Seaborg, *Phys. Review*, 1941, **60**, 913) nor were any applied for possible coincidence loss or density effects. Trials, however, showed that the latter effect was negligible at the activities used in the work.

*Results.* (A preliminary note dealing with the results obtained with a few of these compounds has been published: West, *Nature*, 1950, **165**, 122.)—The results obtained for twelve compounds are summarised in Table 1. Only one set of results has been presented for each substance, although, in general, at least two separate runs were made. Since the separation fractions were made up to definite volumes in all cases (25 or 50 c.c.) the activities listed under the headings " complex " and " $Co^{++}$ " are those of aliquot samples, either 10 c.c. or 20 c.c. In the calculation of % exchange, where necessary, allowance has been made for incomplete extraction of cobalt salt. This has been determined by standard extractions using only a sample of the radioactive cobalt salt, and the results are given under the activity heading, enclosed in parentheses thus (1 200). The smaller figure represents always the activity left in the solvent layer which normally would contain the complex after extraction, and the larger is that of the bulk of the cobalt activity. The activity expected in the complex after complete exchange is obtained by dividing the total standard activity in the ratio of the concentrations of reacting species. In most cases here, since both concentrations are 0·017M, the " complete-exchange " activity is taken as one half of the total standard activity. For bis(salicylideneaniline)cobalt and bis(salicylidene-*m*-toluidine)cobalt, allowance has been made for incomplete extraction of the complex. The amounts of complex in the chloroform extracts (determined as above with an accuracy of about 5%) for the two compounds are given below :

| | Co as $Co_3O_4$ | |
|---|---|---|
| Salicylideneaniline | 5·5 mg. (mean of 3) | Maximum for complete extraction in each case, 6·8 mg. |
| Salicylidene-*m*-toluidine | 5·6 mg. ,, | |

The complex activities in both cases were increased in the ratio maximum/amount found.

In other cases, although the extraction of a complex was never quite complete, the results at a series of time intervals clearly indicated whether exchange had occurred in less than 50 seconds, did not occur at all, or was proceeding slowly. Consequently, since only qualitative

TABLE 1. *The exchange of cobalt ions with cobalt complexes.*

| Compound | Solvent | Temp. | Activity in complex (counts/min.) | Activity in $Co^{++}$ (counts/min.) | Reaction time | Exchange, % |
|---|---|---|---|---|---|---|
| *Cobaltous* | | | | | | |
| Bis-salicylaldehyde | A | 20° ± 0·1° | 123 | 122·0 | 40 secs. | 103 |
| | | | (0·0 | 238·1) | | |
| Bis-salicylideneaniline | B | 20 ± 0·1 | 65·8 | 87·9 | 50 secs. | 102 |
| | | | 66·3 | 90·4 | 6 mins. | 103 |
| | | | 63·4 | 88·1 | 68 mins. | 99 |
| | | | (0·0 | 159·3) | | |
| Bis-salicylidene-*m*-toluidine | B | 20 ± 0·1 | 86·6 | 137·0 | 45 secs. | 95 |
| | | | 88·1 | 137·8 | 2 mins. | 97 |
| | | | 85·6 | 131·2 | 3 mins. | 94 |
| | | | (0·0 | 222·2) | | |
| Bis-salicylidene-*o*-anisidine | B | 20 ± 0·1 | 73·7 | 134·0 | 40 secs. | 74 [d] |
| | | | 70·0 | 137·7 | 2 mins. | 70 [d] |
| | | | 76·7 | 129·3 | 29 mins. | 76 [d] |
| | | | (3·9 | 188·4) | | |
| Bisacetylacetone ($2H_2O$) | B | 20 ± 0·1 | 90·3 | 104·2 | 50 secs. | 92 [d] |
| | | | 91·2 | 108·9 | 1·5 mins. | 93 [d] |
| | | | 94·6 | 100·1 | 2 mins. | 96 [d] |
| | | | (0·0 | 196·6) | | |
| Bisguaiacol | C | ~20 | 25·7 | 215·0 | 5 mins. | 19 |
| | | | 107·9 | 135·6 | 1 hr. | 80 |
| | | | ( | 269·4) | | |
| Bis-salicylaldoxime (brown amorphous product) | A | 20 ± 0·1 | 20·0 | 228·7 | 3 mins. | 15 |
| | | | 19·9 | 223·0 | 6 mins. | 15 |
| | | | 18·3 | 232·0 | 60 mins. | 14 |
| | | | (0·0 | 258·0) | | |
| | | 45 ± 0·1 | 29·8 | 220·7 | 10 mins. | 23 |
| | | | 32·7 | 224·9 | 90 mins. | 25 |
| | | | 35·4 | 220·5 | 240 mins. | 27 |
| | | | (0·0 | 259·3) | | |
| (Preformed complex) | B | 20 ± 0·1 | 186·8 | 191·1 | 18 mins. | 99 [e] |
| | | | 179·7 | 181·1 | 40 mins. | 99·6 [e] |
| | | | 196·2 | 190·9 | 60 mins. | 102 [e] |
| | | | 199·0 | 184·7 | 120 mins. | 104 [e] |
| | | | 191·5 | 183·9 | 180 mins. | 102 [e] |
| Phthalocyanine [a] | B | 30 ± 0·1 | 8·9 | 530·7 | 17 hrs. | 0·4 |
| | | | 10·3 | 534·1 | 109 hrs. | 0·8 |
| | | | (8·0 | 532·9) | | |
| Phthalocyaninetetrasulphonic acid derivative [b] (ammonium salt) | D | 30 ± 0·1 | 3·6 | 1748·4 | 13 days | −0·4 |
| | | | (7·3 | 1799·9) | | |
| Pyrromethene complex | B | 30 ± 0·1 | 111·9 | 1105·6 | 62 mins. | −2 |
| | | | 138·0 | 1091·5 | 3·2 hrs. | 4 |
| | | | 203·0 | 1026·0 | 11·2 hrs. | 16 |
| | | | 349·1 | 885·3 | 37·4 hrs. | 40 |
| | | | 463·5 | 769·4 | 68·2 hrs. | 61 |
| | | | 529·3 | 712·2 | 93·5 hrs. | 72 |
| | | | (79·0) | 1146·2) | | |
| | | | 56·2 | 503·5 | 50 secs. | 6 |
| | | | 165·8 | 410·9 | 43·5 hrs. | 42 |
| | | | (39·3 | 519·4) | | |
| *Cobaltic* | | | | | | |
| Trisethylenediamine chloride ($6H_2O$) | D | 20 ± 0·1 | 2·0 | 241·4 | 10 mins. | 1·6 |
| | | | −1·1 | 239·7 | 1 hr. | −0·92 |
| | | | −2·0 | 234·0 | 5 hrs. | −1·6 |
| | | | −1·3 | 241·0 | 24 hrs. | −1·7 |
| | | | (0·0 | 238·1) | | |
| Tris-1-nitroso-2-naphthol [c] | B | 25 ± 0·1 | 1·0 | 392·3 | 35 secs. | −0·98 |
| | | | 8·7 | 386·2 | 113 mins. | 4·9 |
| | | | 20·3 | 352·4 | 90 hrs. | 13·7 |
| | | | (2·3 | 397·2) | | |
| | | | 15·4 | 178·6 | 221 hrs. | 23·8 |
| | | | 22·4 | 171·7 | 26 days | 34·6 |
| | | | (0·0 | 194·0) | | |
| Trisacetylacetone | B | 30 ± 0·1 | 122·8 | 1362·7 | 3 days | 3 |
| | | | 139·1 | 1383·2 | 6 days | 5 |
| | | | 153·4 | 1383·6 | 9 days | 7 |
| | | | 185·4 | 1364·1 | 18 days | 12 |
| | | | 203·3 | 1338·3 | 21 days | 14 |
| | | | (101·2 | 1383·2) | | |

(See references on following page.)

information was required, corrections for incomplete extraction of complex were not applied, the only correction made being that for incomplete cobalt salt removal. For the salicylaldehyde, salicylidene-*o*-anisidine, and acetylacetone complexes, where incomplete extraction might be expected to be considerable, the high, constant activities of the complex extracts show that complete exchange has occurred in less than 40 seconds. The low, constant values for the first salicylaldoxime runs may be regarded as evidence for the existence of a small amount of an exchangeable compound.

An apparent "separation-induced" exchange of 6% has been observed for the pyrromethene complex by a series of runs at times of about 30 seconds. This factor has been allowed for in calculating % exchange.

## Discussion

*Bis(salicylaldehyde)cobalt*(II).—Under the conditions of exchange used in this work complete exchange is observed with this compound in times as small as 50 seconds. This points to the existence of ionic bonds between the metal and surrounding ligands when in pyridine solution. The magnetic data available support this conclusion ($\mu = 4{\cdot}5$; Tyson and Adams, *J. Amer. Chem. Soc.*, 1940, **62**, 1228).

*Bis(acetylacetone)cobalt*(II).—Similarly, complete exchange has been observed to occur for this compound in less than 1 minute, and hence the existence of ionic bonds may be inferred here also. A susceptibility of 4·3 $\mu$ given by Mellor and Goldacre (*J. Proc. Roy. Soc. N.S.W.*, 1939, **73**, 233) supports this view.

*Bis(guiacol)cobalt*(II).—The slower rate of exchange observed here has been related in a preliminary communication (West, *loc. cit.*) to the experimental procedure adopted. Exchange proceeding between a solid and a solution will perforce be slow because of the necessity for the exchanging ions to diffuse into the crystal structure of the solid. Again, the magnetic evidence supports the conclusion that ionic type bonds exist in this compound, Mellor and Craig obtaining a value of 4·5 $\mu$ for this substance (*ibid.*, 1940, **74**, 495).

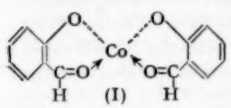
Table 2. *The exchange of metal ions with metal complexes.*

| Exchanging species | Solvent | Result | Ref. |
|---|---|---|---|
| $Mn^{++}$ bisacetylacetone | Methanol | Rapid exchange | *a* |
| $Zn^{++}$ bisacetylacetone | Pyridine | Complete in 30 secs. | *b* |
| $Cu^{++}$ bisacetylacetone | Chloroform | Complete in 2 mins. | *c* |
| $Cu^{++}$ bis-salicylaldehyde | Pyridine | ,, 15 secs. | *d* |
| bis-salicylideneaniline | ,, | ,, 15 secs. | *d* |
| bis-salicylidenemethylamine | ,, | ,, 15 secs. | *d* |
| $Ni^{++}$ bis-salicylaldehyde | Methyl cellosolve | ,, 5 mins. | *e* |
| bis-salicylaldoxime | ,, | ,, 5 mins. | *e* |

*a*, Drehman, *Z. physikal. Chem.*, 1943, **53**, 227. *b*, Leventhal and Garner, *J. Amer. Chem. Soc.*, 1949, **71**, 371. *c*, Ruben, Seaborg, and Kennedy, *J. Appl. Physics*, 1941, **12**, 308. *d*, Duffield and Calvin, *loc. cit.* *e*, Johnson and Hall, *J. Amer. Chem. Soc.*, 1948, **70**, 2344.

*Bis(salicylideneaniline)cobalt*(II).—This substance, together with the *o*-anisidine and the *m*-toluidine derivative, also undergoes complete exchange in reaction times of less than 1 minute, showing that ionic-type bonds are present in these compounds. No magnetic susceptibilities are available for these three compounds.

Similar results to these have been reported by a number of workers with Mn, Cu, Ni, and Zn complexes (see Table 2).



(I)

For each of these six compounds the conventional valency bond structure would indicate that each organic residue was joined by one co-ordinate linkage and one ionic linkage, as, *i.e.*, bis(salicylaldehyde)cobalt(II) (I). However, Syrkin and Dyatkina ("Structure of Molecules and the Chemical Bond," Butterworths, London, 1950, p. 389) have shown that the structure of the somewhat analogous glycine copper compound is best represented by

A, Complex in pyridine, $CoSO_4$ in water. B, Complex in pyridine, cobalt acetate in pyridine. C, Solid compound shaken with aqueous $CoSO_4$ and EtOH. D, Complex in water, $CoSO_4$ in water. *a*, Solutions in pyridine; complex 0·00425M, cobalt acetate 0·0085M. *b*, Complex 0·001M, cobalt sulphate 0·017M in 0·1M-$H_2SO_4$. *c*, Complex 0·00425M; 10 c.c. of this solution mixed with 5 c.c. of 0·0085M-cobalt acetate. *d*, 100% exchange assumed since allowance not made for incomplete extraction of complex. *e*, Standard was not obtained; results are calculated on the basis of expected activity at 100% exchange = ½ × (sum of chloroform + water activities). *f*, This value for induced exchange has been used to correct every other % exchange value for this compound.

resonance between a number of structures with the result that no difference is to be observed between either the Cu–O or the Cu–N bonds. Hence each bond must be associated with partial ionic character. Hückel ("Structural Chemistry of Inorganic Compounds," Vol. I, transl. Long, Elsevier, Amsterdam, p. 141), on the other hand, allows a fixed covalent linkage for the Cu–N bond but assumes that the Cu–O linkage represents a transition to a covalent linkage from a simple ionic bond with the probability, however, that this Cu–O linkage can dissociate in water.

For the cobalt complexes Syrkin and Dyatkina's resonance treatment seems the better of the two because the high electronegativity of O and N leads to considerable ionic character in the Co–N or Co–O co-ordinate linkages and some covalent character in the Co–O links between Co and the phenolic OH groups. Thus one may explain the easy exchange of the central metal atom by another without the necessity of postulating the rupture of what one might expect, *a priori*, to be a strong Co–N or Co–O co-ordinate linkage, since these bonds must now be considered to possess considerable ionic character.

The magnetic susceptibilities of the first three complexes have pointed to a tetrahedral configuration for each compound, and since this is expected with ionic type bonding it is probable that the salicylideneaniline-type complexes have a tetrahedral configuration also.

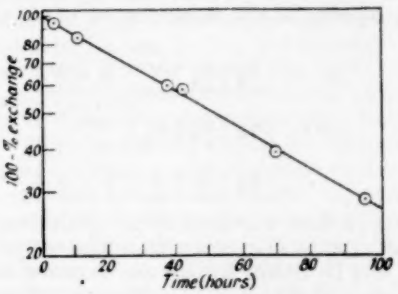
*Bis(salicylaldoxime)cobalt*(II).—In the work on the brown, amorphous sample of this complex a small, constant amount of exchange was observed during 60 minutes at 20°, but a slightly higher % exchange was observed at 45° with only a slight further increase in 4 hours. Mellor and Craig (*loc. cit.*) have shown by magnetic measurements that a square, coplanar configuration is to be expected for this compound, with expected strong covalent bonding. These authors also showed that a similar configuration existed in the corresponding nickel derivative (*ibid.*, 1940, **74**, 475). Hence, if exchange were observed, it would be expected to be slow. Johnson and Hall (*loc. cit.*), however, showed that the nickel complex exchanged completely within 5 minutes in 2-methoxyethanol—a fact which points rather to bonds with considerable ionic character. In view of the tendency of many cobaltous salicylideneamine derivatives to absorb oxygen (Calvin *et al.*, *J. Amer. Chem. Soc.*, 1946, **68**, 2254, 2257, 2263, 2267, 2273, 2612), a second run was carried out in which the cobalt complex was pre-formed in pyridine solution under an atmosphere of nitrogen to avoid possible oxygen contamination, and complete exchange was observed within 18 minutes.

As mentioned on p. 3117, the yellowish solution of complex, prepared in the absence of oxygen, became deep brown on exposure to air or on oxygen being bubbled through the solution. It seems probable therefore that the brown amorphous complex used in the first experiments was an oxygenated derivative containing a small amount of pure bis(salicylaldoxime)cobalt, the latter substance being able to exchange quickly with cobalt ions while the former substance was not. If the analytical figures are recalculated for a molecule of bis(salicylaldoxime)cobalt containing half an atom of oxygen, much better agreement is obtained with the carbon value for the amorphous product than previously; thus, Found: C, 49·4, 49·4; H, 3·7. Calc. for $Co(C_7H_6O_2N)_2\frac{1}{2}O$: C, 49·6; H, 3·6%. In the first run, the higher temperature could be causing slight removal of oxygen from the complex, leading to an increase in the amount of "free" bis(salicylaldoxime)cobalt. This explanation seems more in agreement with the existing facts than that given in the first instance (West, *loc. cit.*). It thus appears necessary to presume that both the nickel and the cobalt derivatives contain bonds with considerable ionic character when in solutions of methoxyethanol and pyridine respectively.

*Bis-(4 : 4'-dicarbethoxy-3 : 3'-5 : 5'-tetramethyldipyrromethene)cobalt*(II).—This compound shows slow exchange proceeding during many hours. A conventional plot of log (100 − % exchange) against time (see Fig.) shows that the appearance of activity is following the expected first-order law with $t_{\frac{1}{2}} \simeq 51{\cdot}5$ hours. There exists no direct evidence about the configuration of this compound, although Mellor (*J. Proc. Roy. Soc. N.S.W.*, 1940, **74**, 138) has suggested that it should be allotted a square, coplanar configuration. Because of the steric interaction of the α-methyl groups, metal complexes of this type will not be completely planar but the metal–nitrogen bonds will be distorted from the coplanar position with a consequent change in bond type from the expected covalent $dsp^2$ planar

type to bonds with considerable ionic character. This has been shown to occur with the nickel derivative, which has a moment of 3·2 Bohr magnetons (Mellor and Lockwood, *ibid.*, 1940, **74**, 141). It is probable, in view of this result, that a tetrahedral configuration may be applicable here in spite of the *X*-ray evidence for the planarity of the four pyrrole nitrogen–Ni bonds in nickel phthalocyanine (Robertson, *J.*, 1937, 219). However, the occurrence of slow exchange for the cobalt complex seems to imply that the bonds between cobalt and the pyrrole nitrogens have considerable covalent character, and this would agree with an essentially square planar structure for the complex with methyl group interaction causing " out of plane " distortion of the Co–N bonds leading to a configuration somewhere between planar and tetrahedral and containing Co–N bonds with partial ionic character. Further exploratory results not given in this paper seem to show that $t_{\frac{1}{2}}$ is not independent of concentration, so the collision mechanism of exchange is probably operating here rather than an ionisation and recombination.

*Cobaltous Phthalocyanine.*—Klem and Klem (*J. pr. Chem.*, 1935, **143**, 82) have found a value of 2·16 μ for the magnetic moment of this compound, leading to the assumption of a square, coplanar formulation with strong $dsp^2$ covalent bonds. No evidence for exchange has been obtained for either the simple complex in pyridine or the water-soluble sulphonic acid derivative in aqueous 0·1M-sulphuric acid. The bond here may, therefore, be classified as strong covalent. However, these bonds may not necessarily be as strong as, say, the




*Cobalt pyrromethene exchange at 30° in pyridine solutions, 0·017M.*

Co–N linkages in cobaltic ethylenediamine complexes (see below) in spite of the observed lack of exchange. Ruben and his co-workers (*J. Amer. Chem. Soc.*, 1942, **64**, 2297) have shown that ferric ions do not exchange with ferrihaem in which the iron is presumably bound by ionic bonds according to magnetic measurements (Pauling and Coryell, *Proc. Nat. Acad. Sci.*, 1936, **22**, 159). These workers suggest that the fused-ring system present in such compounds would in some way prevent exchange even when bond considerations should permit it. Probably the occurrence of such a strongly resonating structure as the phthalocyanine or porphyrin ring can help to stabilise any metal compounds formed to a greater extent than is possible with other complex-forming molecules.

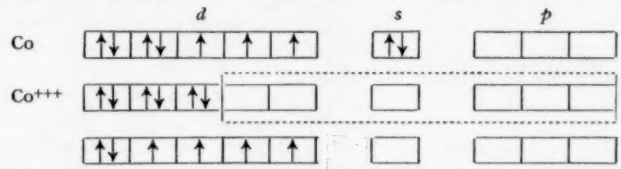
*Tris(ethylenediamine)cobalt*(III) *Chloride.*—No exchange was observed with this compound in water during 24 hours, in agreement with Lewis and Coryell (*J.*, 1949, S 386), and this fact points to strong covalent bonding. Flagg (*J. Amer. Chem. Soc.*, 1941, **63**, 557) and McCallum, Hoshowsky, and Holmes (*Canadian J. Res.*, 1949, **27**, *B*, 258) have shown that other cobaltic complexes of this type do not exchange with cobaltous ions over long periods.

*Tris-(1-nitroso-2-naphthol)cobalt*(III).—The occurrence of exchange, even though slight, is doubly interesting in the case of this compound because, first, we have a very stable cobaltic compound undergoing exchange with cobaltous ions, and secondly, in the process an electron transfer must occur between the free cobaltous and the complexed cobaltic ions. It is, no doubt, more correct to speak in every case dealt with in this paper, not of free cobaltous ions but rather $[Co(py)_4]^{++}$, or some similar entities. The very slow rate observed is to be expected if covalent bonding with some ionic character is present in this complex, it being borne in mind that exchange in this case is a two-stage process. With the

1 : 2-naphthaquinone 1-oxime formula given by Hückel (*loc. cit.*, p. 145) for the complex, a definite contribution of ionic valency bond structures to the resonance stabilisation of the complex must be expected because of the high electronegativity of oxygen. Consequently, the assumption of partial ionic character for the Co–O bonds in this complex seems justified in view of the evidence for exchange.

*Tris(acetylacetone)cobalt*(III).—Cambi and Szego (*Ber.*, 1931, **64**, 2591) quote a value for the magnetic susceptibility of this compound determined by Ishiwara (*Rep. Tohoku Univ.*, 1916, **5**, 53; not available to the author). The value of 4·6 Weiss magnetons is 0·93 Bohr magneton in the more usual modern interpretation, and this has been taken by Cambi and Szego to indicate diamagnetism for the compound. Sugden (*J.*, 1943, 328) has quoted a value of 0·00 μ for this substance without reference to its source. The author has been unable to trace any further reference to a magnetic measurement. In view of the evidence for exchange it appears that the cobalt–ligand bonds must possess some ionic character and are not, therefore, as strongly covalent as would be implied by the assumption of diamagnetism for this substance. Syrkin and Dyatkina (*op. cit.*, p. 391) have accounted for the properties of diamagnetic tris(acetylacetone)aluminium and of the paramagnetic ferric compound by assuming that $sp^2$ hybridization occurs with the formation of three ordinary electron-pair bonds by the replacement of the acidic hydrogen of the hydroxyl group present in the tautomeric reactive form of acetylacetone.

On the other hand, conventional $d^2sp^3$ octahedral bonds would cause the cobalt complex to be diamagnetic, as also would the use of the three unpaired electrons of a cobalt atom:




However, if three *d* orbitals of the cobaltic ion are utilised for covalent bond formation, giving a $d^3$ hybridization, the constitution of the complex is again similar to the postulate of Syrkin and Dyatkina, leaving one unpaired electron, which should give the complex a magnetic susceptibility of 1·73 μ (Pauling, " Nature of the Chemical Bond," Cornell, 2nd edn., 1940, p. 113). If the value determined by Ishiwara is significant, and several other instances of low paramagnetic susceptibilities have been reported (Cambi, Ferrara, and Colla, *Gazzetta*, 1936, **65**, 1162) for compounds where the value 0·00 μ was expected, then it may point to a state of bonding similar to that suggested. Since $d^3$ would tend to be weaker than $sp^2$ bonds, the occurrence of exchange is possibly explained.

On the other hand, the high electronegativity of oxygen could be expected to lead to some ionic character for each Co–O bond; consequently, the exchange seems not incompatible with a conventional octahedral complex. Further information obtained after investigation of other cobaltic complexes seems highly desirable in order to clarify this problem and also that of the 1-nitroso-2-napthol complex.

Microanalyses were carried out by Mr. R. T. Howard and by the C.S.I.R.O., Microanalytical Laboratory, Melbourne.

The author is indebted to Dr. S. W. Pennycuick for his interest and encouragement in this work, and gratefully acknowledges the assistance of Mr. H. R. Marston, F.R.S., Chief of the Division of Biochemistry and General Nutrition, C.S.I.R.O., Adelaide, and Dr. T. Oddie, Tracer Elements Section, C.S.I.R.O., Melbourne, in obtaining the $^{60}$Co used in this work and for discussions on counting technique. The Commonwealth Research Fund is thanked for grants towards the cost of equipment, and Dr. J. P. E. Human for the construction of the apparatus used in measuring exchanges in the absence of oxygen.

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## 598. *Studies on Bond Type in Certain Cobalt Complexes. Part II.* The Constitution of Cobalt Complexes with Salicylaldehyde Anils.*

By B. West.

Efforts have been made toseparate mixtures of bis(salicylidene aniline)-cobalt and cobalt acetate in pyridine solution by chromatography on alumina and similar surfaces and by use of an ion-exchange resin. A break-up of the complex on these surfaces has been demonstrated by the use of the $^{60}$Co-labelled complex. A partial separation has been attempted by the use of an electro-diffusion cell but some cobalt from the complex tends to move to the cathode. Measurements of the absorption spectra of the complex and the base in ethyl alcohol–water mixtures have shown that an ionization may occur in the presence of water, as do conductivity measurements taken in pyridine–water mixtures. These results point to the weakness of binding of the cobalt atom in such complexes, all the bonds apparently having considerable ionic character.

In Part I * some conclusions were drawn as to the type of bonding in a group of cobaltous compounds of the inner complex type, where extremely rapid exchange was observed to occur between cobaltous ions in pyridine solution and the cobalt of the complexes. It had been tacitly assumed in discussing the results presented in that paper that the separation method used (extraction with chloroform and water) had no effect on the exchange observed. Several authors, however (*e.g.*, Garner and Meier, *J. Amer. Chem. Soc.*, 1951, **73**, 1894; Prestwood and Wahl, *ibid.*, 1948, **70**, 880), have shown that an apparent exchange can be induced by the separation procedure used in studying an exchange reaction when precipitation methods of separation are employed. Calvin and Duffield (*J. Amer. Chem. Soc.*, 1946, **68**, 557) have reported that keeping an extraction mixture of bis(salicylaldehyde)ethylenediaminecopper and radioactive copper acetate for 15 minutes did not cause a greater % exchange than was expected for the time at which the exchange was stopped by addition of chloroform and water for the purpose of separation. However, this type of experiment does not show whether or not an induced exchange occurs on the *addition* of the solvents for extraction purposes. In the cobalt exchange results reported in Part I (*loc. cit.*) evidence was obtained for a constant exchange of 6% occurring whenever the solvent extraction process was used for bis-(4 : 4′-dicarbethoxy-3 : 3′ : 5 : 5′-tetramethyldipyrromethene)cobalt(II), a separation made after 40-seconds reaction time always giving a value much higher than the slow rate of exchange would allow, at the end of so short a time interval. This was used to correct all other values for % exchange at other time intervals. For bis(salicylideneaniline)-cobalt and similar compounds where exchange was observed to be complete after times as short as 30 seconds, " standard " exchanges similar to that carried out for the pyrromethene complex were impossible. Consequently, attempts were made to effect a separation of the reaction components by methods other than extraction procedures with a view to determine whether water or chloroform could cause exchange to occur when added to a reaction mixture.

A separation technique involving precipitation of one or other component could not be found, since most normal insoluble cobalt salts such as the phosphate, carbonate, etc., could not be obtained in pyridine solution. Organic metal complexes also would themselves be soluble in pyridine and could not be employed for that reason. There may exist a difficultly soluble compound of cobalt which could be formed in pyridine, but it is almost certain that water would have to be added in the process, and would, of necessity, invalidate the technique. Attention was then turned to physical methods such as diffusion and chromatographic adsorption.

It was decided to use bis(salicylideneaniline)cobalt as the complex in all these investigations, mainly for the reason that no other evidence, such as a magnetic susceptibility measurement, was available to enable a comparison of the bond type determined by that

* Part I, preceding paper.

method and by the exchange method to be made so that a decision as to the effect of the separation procedure on exchange was possibly of more urgent importance with this compound. In order to judge the separation that could be effected by the various procedures tried, a sample of the complex was synthesised containing radioactive cobalt. Activity determinations could then give immediate information as to the distribution of the complex.

*Chromatographic Adsorption.*—1. *Alumina.* Alumina columns, prepared as described on p. 3127, were used to study the adsorption of cobalt ions and of bis(salicylideneaniline)-cobalt, both from pyridine solution. By using cobalt acetate solutions labelled with $^{60}Co$ it was found possible to adsorb more than 99·5% of Co on alumina columns at 0·003M. Labelled bis(salicylideneaniline)cobalt, however, showed that the complex, at 0·017M, could only be eluted from similarly constructed columns to a maximum extent of 58·7%. Lower elution percentages were obtained with lower concentrations of complex or with slight increases in the amount of alumina in the column. In chromatography of solutions of the complex, a pink band similarly placed to that obtained with cobalt acetate was noticed which was presumably due to adsorbed $[Co(py)_x]^{++}$ ions in both cases. Also the colour of the eluate from the complex adsorptions was bright yellow rather than the yellow-orange associated with pyrridine solutions of the complex. The free base gives an intensely yellow solution in pyridine (and other organic solvents), so all the evidence points to at least a partial break-down of the complex on the column, leading to the adsorption of a portion of the cobalt and elution of the remainder of the complex together with the excess of free base. Possibly hydrogen ions present in the column may replace the cobalt atoms in the complex molecules, although strict precautions were taken with the alumina used to remove the last traces of acid. On the other hand, water molecules which would still, in all probability, be present in the alumina could react with the complex, giving hydrated cobaltous ions and base ions. The latter possibility seems the more probable in view of the spectroscopic and conductimetric evidence to be mentioned below.

In any case a separation procedure using this technique would not preclude the possibility of an induced exchange occurring on the alumina surface since the complex can break up into its components, at least partly, when passed down such a column.

2. Further attempts using magnesia, silica, and talc gave similar results to those obtained with alumina.

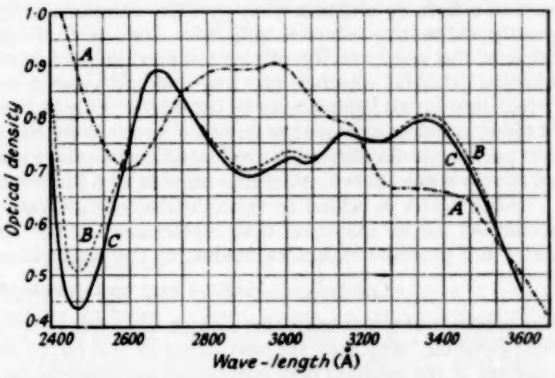
3. *Ion-exchange resin.* A sample of a cationic exchange resin (Amberlite IR-100, Na salt) was then tried, the column technique described below being used. When used directly as obtained, scarcely any separation of the two compounds could be obtained. The complex was 100% eluted at 0·017M, and cobalt acetate at 0·0033M was eluted to the extent of about 91%. The resin was then crushed to a fine powder, in order to increase the surface area, and a hold back of 79% was obtained with a pyridine solution of cobalt acetate. However, the complex could only be eluted to the extent of 57%, and from the bright yellow colour of the eluate it seems probable that, again, break-down of the complex occurs on this column, probably owing to an exchange of the sodium ions on the column for the cobalt of the complex. Consequently, the use of this method suffers from the same difficulty as the alumina column method in that the possibility of a surface exchange is not eliminated.

4. *Electro-diffusion.* An apparatus was constructed for carrying out separations by electro-diffusion (see p. 3127), as the times required to accomplish separations by simple, flow-diffusion were expected to be much greater than the possible completion of exchange with this compound. By using 200 v D.C. across the apparatus, cobalt could be made to pass into the cathode compartment from pyridine solutions of cobalt acetate to the extent of about 3% in 20 minutes. The complex also showed a tendency for activity to enter the cathode, while a distinct yellow colour could be observed on the anode compartment side of the sintered-glass disc separating the anode from the central compartment. Evidently, in this case also, break-down of the complex can occur on passage of an electric current, so the probable ionic type bonds already suggested for this compound may allow migration of the appropriate ions. It did not seem possible, in consequence of this, to use the method as a separation procedure.

In spite of the failure to find a separation technique which did not, of itself, cause the

break-down or ionization of the complex, this especial fact is extremely important in that it shows a pronounced weakness of the bonds in this particular complex compound. Thus, very rapid exchange may be expected to occur in any case, and a technique such as that developed by Barker (reported by Betts *et al.*, *J. Amer. Chem. Soc.*, 1950, **72**, 4978) would seem to be ideally suited for investigating the exchange of compounds of this type.

As a further aid in elucidating the effect of water on the constitution of the complex, the ultra-violet absorption spectra of the complex and base were obtained in absolute ethyl alcohol and in aqueous alcohol at various percentages of water. Ethyl alcohol was used instead of pyridine for the determination of spectra because of the strong tendency of pyridine solutions of the complex to creep over the top of the only cells available for the instrument and to flow down the optical faces, thus causing fluctuations in optical density readings. This occurred even when the cells were covered with the plastic caps supplied by the manufacturer. The concentration of salicylideneaniline used was $8 \times 10^{-5}$M, and that of the cobalt complex formed from the base, $4 \times 10^{-5}$M. If an ionization should occur on the addition of water to the complex solution, then complete ionization would be shown by the similarity of the optical density–λ curves obtained for base and complex solutions of the above concentrations at some particular percentage of water. Since two molecules of salicylideneaniline are present in one molecule of the cobalt complex, the




*Absorption spectra of :*
*A*, *Bis(salicylideneaniline)cobalt*(II), $4 \times 10^{-5}$M *in absolute ethyl alcohol.*
*B*, *Salicylideneaniline*, $8 \times 10^{-5}$M *in* 10% *aqueous ethyl alcohol.*
*C*, *Bis(salicylideneaniline)cobalt*(II), $4 \times 10^{-5}$M *in* 10% *aqueous ethyl alcohol.*

liberation of these molecules gives a solution of base twice the strength of the complex solution. As is shown in the figure, this has occurred when 10% of water is present. Also in the figure is shown the curve for the complex in absolute alcohol. Salicylideneaniline gives essentially the same curve both in absolute alcohol and in 10% aqueous alcohol.

A weak absorption band for the cobalt ion occurs at much longer wave-lengths (*ca.* 5000 Å); consequently, it is not expected to interfere with the absorption due to the free base ion between 2600 and 3500 Å. Therefore, the disappearance of the band due to the cobalt complex upon the addition of water and the appearance of a curve similar to that of the free base is good evidence that a break-down (presumably due to ionization) of the complex is occurring with the liberation of free base ions. Further, since the curves for complex and base at 10% of water are essentially the same, *i.e.*, each contains the same amount of free base or ion, at this stage complete ionization must have occurred.

Since it was not possible to show directly that a similar state of affairs existed for pyridine–water systems because of the experimental difficulty mentioned above, a series of conductivity measurements were made on pyridine solutions of bis(salicylideneaniline)cobalt containing various percentages of water, and the results compared with similar measurements on similarly prepared pyridine–water mixtures. No claim is made for the absolute accuracy of the results given in Table 1 because the purpose of the measurements was solely to obtain a demonstration of ionization, and this could be gained by a comparison of the two sets of conductivity data. The solutions used in each case were prepared, and their conductivities measured, in exactly the same manner (see p. 3128), but the limitations of the measuring system were too great to allow any but semiquantitative values to be obtained. The

results show quite definitely, however, that a much greater increase in conductivity occurs for pyridine solutions of the complex to which water is added than is found when water is added to pyridine alone. The higher value of $\kappa$ obtained for the complex solution in pure pyridine than for solvent alone is probably not significant owing to the difficulty of measuring such high resistances accurately with the equipment available; although, in view of the evidence of migration of cobalt obtained in the experiments on electro-diffusion, some ionization may occur, even in the pure solvent.

TABLE 1

| | $H_2O$, % | 0 | 2 | 5 | 10 | 15 | 20 | 30 |
|---|---|---|---|---|---|---|---|---|
| $10^6\kappa$ | Complex solution ..... | 0·085 | 0·47 | 0·98 | 1·7 | 4·2 | 7·5 * | 8·1 |
| | Solvent only ..... | 0·042 | 0·085 | 0·22 | 0·47 | 0·89 | 1·7 | 3·0 |

Complex concentration = $8{\cdot}5 \times 10^{-3}$M.
* 20% or more of water causes precipitation of the complex from solution.

## DISCUSSION

It appears, therefore, from these experimental results that the introduction of water to a pyridine solution of bis(salicylideneaniline)cobalt can bring about the formation of " dissociated " ionic species. The word " dissociated " has been used because the adsorption of cobalt by alumina from pyridine solutions of the complex seems to imply that in pure pyridine the complex molecules may be formulated as undissociated, neutral, ion triplets, the conductivity of the complex in pure pyridine being so small that it is extremely doubtful whether any free ions are present in solution, *i.e.*, the bonding of the cobalt to the two ligands may be considered as being essentially ionic. With such a weak binding, cobalt is expected to be easily removed by the active alumina. Probably at least two pyridine molecules are co-ordinated to the cobalt ion in each complex molecule, making up the total possible co-ordination number of 6 for bivalent cobalt (see also p. 3127).

When water is added to the solution, the increase in conductivity observed may be accounted for by assuming that replacement of the chelating ligands can take place by water and pyridine molecules, leading to the production of various ionic ionic species, *e.g.*

$$\mathrm{Co(chel)_2} + x\mathrm{H_2O} + y\mathrm{py} \rightleftharpoons [\mathrm{Co(H_2O}_z)\mathrm{py}_y]^{++} + 2\mathrm{chel}^-$$

The presence of water may also exert a dielectric effect and aid in dissociation, whereas in pure pyridine, although the formation of $[\mathrm{Co\,py_4}]^{++}$ ions is possible, the low dielectric constant of the medium may prevent actual dissociation of the molecule, *i.e.*, an ion-triplet formulation could be expected, as mentioned previously.

Thus the observation of rapid exchange with compounds of this type in pyridine (Part I, *loc. cit.*) when a separation procedure involving water is used would probably also be noticed if a separation method not involving the use of water could be devised. The bonds in the complex must presumably be regarded as possessing considerable ionic character and therefore not presenting a strong barrier to replacement by other cobalt ions present in solution. If one regards a free cobalt ion in pyridine as being $[\mathrm{Co\,py_4}]^{++}$ then easy replacement is to be expected by collision with the postulated ion triplet whether this be $\mathrm{Co\,py_2\,chel_2}$ or $[\mathrm{Co\,py_4}]^{++}(\mathrm{chel})_2$.

It is noteworthy in this regard that a series of unpublished experiments on the chromatography of benzene, nitrobenzene, and ethyl alcohol solutions of bis(salicylideneaniline)-cobalt on alumina have shown analogous effects to those obtained with pyridine solutions, *i.e.*, some cobalt is adsorbed and free base plus complex is eluted. In each case the conductivities of the solutions used are not detectably different from those of the pure solvents. For these solutions, and especially for benzene, no " solvation " is expected similar to that possible with pyridine. This seems to strengthen the view that the complex must be regarded as having cobalt–ligand bonds with strong ionic character.

With regard to the spectroscopic data obtained in ethyl alcohol–water solutions for the salicylideneaniline complex, although the change in spectrum may be interpreted as being due to an ionization of the complex in the presence of water, an alternative explanation might be possible in view of the work of Mellor and his co-workers (*J. Proc. Roy. Soc. N.S.W.*, 1944, **78**, 70). In an investigation of the spectra of nickel complexes of the chelate

type, he has shown that complexes possessing paramagnetic susceptibilities, and hence tetrahedral ionic structures, show absorption spectra similar to those of the bases from which they are obtained. Square complexes with presumed covalent bonding have spectra somewhat different from those of the free bases. The spectrum of the complex being investigated here is quite different from that of the free base, and it is only on the addition of water that a change to the base spectrum occurs. Consequently, if Mellor's conclusions are of wide application, they may point to the change from a square, coplanar configuration of bis(salicylideneaniline)cobalt when in absolute alcohol to a tetrahedral configuration in the presence of water. The former configuration would allow the compound to form covalent type bonds between the metal and the surrounding ligands, while the latter would imply the formation of bonds, mainly ionic in character, giving the complex in aqueous media the form of a neutral ion triplet. The bonds in the square form would necessarily be weak in order to allow the change in bond type to occur easily.

This suggestion could be related to the situation in pyridine if one were to assume that complexes of the type Co $py_2$ $chel_2$ existed in solution. The pyridine molecules could then be regarded as being attached to the cobalt above and below the plane of the ordinary molecule. In this connection Tyson and Adams (*J. Amer. Chem. Soc.*, 1940, **62**, 1228) have mentioned the difficulty of deciding between the two alternative configurations for bis-salicylaldehyde)cobalt even though their magnetic data may be interpreted perhaps more reasonably in terms of the tetrahedral structure. Certainly, the *X*-ray examination of many copper and nickel derivatives of similar type shows the widespread occurrence of planar forms (Stackelberg, *Z. anorg. Chem.*, 1947, **253**, 136; Cox *et al.*, *J.*, 1935, 731; 1936, 129, 1775). An *X*-ray crystallographic study of cobalt complexes of the chelate type seems necessary to provide the final explanation. The fact, however, that no isomers have been reported for cobalt complexes of the type under discussion seems to point to the tetrahedral structure being more probable. It is hoped later to present some chemical evidence to support a projected tetrahedral structure.

## Experimental

1. The alumina used in the experiments was "B.D.H. Chromatographic Alumina" which was washed in running distilled water for 24 hours or more before use and dried at 250°. Columns were prepared by slurrying the oxide with pyridine and pouring it into a glass Buchner funnel, 4 cm. wide and 10 cm. deep, having a perforated, procelain plate covered with filter-paper of appropriate diameter on the bottom. The height of the column used varied with the experiment, but was in general 2—3 cm. Some of the results obtained are given below, and suffice to illustrate the results obtained with the various materials. Columns of this nature were prepared with each of the other adsorbents tried. Gentle suction was applied to aid the movement of the solutions, and in each case elution of the column was carried out with the aid of pyridine or chloroform. The removal of adsorbed $Co^{++}$ was carried out with warm, dilute nitric acid in all cases, a few c.c. being poured through the column until no further evidence of pink $Co^{++}$ could be seen. A check of the total activity recovered against a standard activity previously prepared guarded against any loss. All counting was carried out in 20-c.c. glass-skirted Geiger counters, with a scale-of-100 counting unit, and corrections for background were applied in each case.

2. Magnesia and silica were both pure reagent-grade samples, washed as above and activated at 300°.

3. The Amberlite IR-100 was treated in bulk with 10% sodium chloride solution, washed thoroughly with distilled water, then boiled with pyridine and kept in pyridine for at least 12 hours before use.

4. *Electro-diffusion.*—The apparatus consisted of a right-angled U-tube of 2·0 cm. internal diameter with two sintered-glass plates of pore size 3, fused parallel to each other inside the base of the U enclosing a volume of 20 c.c. between them. A capillary tap was attached to the bottom of this central compartment, and a filling tube or 1 cm. diameter to the top. So that pressure differences between the side arms and the central compartment could be equalized, this filling tube was made to project to the same height as each side arm. In operation, the side arms were filled to a definite mark with 1% potassium chloride solution, the central compartment meanwhile being stoppered to prevent flow through the glass membranes. The stopper was

| Run | Solution used | Column | Eluate activity (counts/min.) | Eluting solvent | Hold back activity (counts/min.) | Standard (counts/min.) | Remarks |
|---|---|---|---|---|---|---|---|
| 1 | Cobalt acetate in pyridine (0·0033M), 5 c.c., +5 c.c. of pyridine | $Al_2O_3$, 2 cm. high | 8·7 | $CHCl_3$, 10 c.c. | 526·8 | 529·0 | 99·5% Hold back |
| 7 | Complex in pyridine (0·017M), 5 c.c., +5 c.c. of pyridine | ,, | 123·9 | ,, | 100·5 | 223·1 | 45% Hold back |
| 8 | ,, ,, | ,, | 127·7 | ,, | 89·7 | 217·5 | 58·7% Eluted |
| 11 | ,, ,, | IR-100 resin, uncrushed, 2 cm. high | 227·0 | Pyridine, 8 c.c. | 0·0 | 223·8 | 100% Eluted |
| 12 | Cobalt acetate in pyridine (0·0033M), 5 c.c., +5 c.c. of pyridine | ,, | 356·2 | ,, | (Not counted) | 385·2 | 92% Eluted |
| 15 | Cobalt acetate in pyridine (0·0033M), 5 c.c., +5 c.c. of pyridine | IR-100 resin crushed | 23·7 | Pyridine, 10 c.c. | 517·6 | 529·0 | 4·4% Eluted |
| 16 | Complex in pyridine, as for 7 | ,, | 66·5 | ,, | (Not counted) | 223·8 | 30% Eluted |
| 22 | ,, ,, | MgO, 3-cm. column | 221·8 | ,, | ,, | 307·4 | 72% Eluted |

then removed, and 20 c.c. of the appropriate solution were run in to the central compartment until the level in the filling tube corresponded with that in the side arms. A bright platinum electrode was then immersed in each side arm, and connection made to the current supply. After the time allowed for diffusion had elapsed, each section of the vessel was emptied, the solutions were made up to similar volumes, and aliquots counted. The entrance of cobalt ions to the cathode compartment was accompanied by the formation of long tendrils of blue $Co(OH)_2$, due to the hydroxyl ions produced by electrolysis in the cathode compartment. This was dissolved in dilute hydrochloric acid when making up the solution from this compartment, preparatory to counting. It was found extremely difficult to prevent streaming occurring from the solutions in the side arms into the central compartment. However, the method of filling mentioned above seemed to give the best results possible with the apparatus. Runs carried out with low voltages (10 v) gave little evidence for diffusion except after periods of the order of 1½—2 hours. The final runs, therefore, were carried on for 20 minutes at an applied voltage of 200 v D.C. The results are given in Table 2.

TABLE 2.

| Run | Solution used | Anode (counts/min.) | Central (counts/min.) | Cathode (counts/min.) | Time, mins. | Voltage, v | Remarks |
|---|---|---|---|---|---|---|---|
| 5 | 0·017M-Cobalt acetate, 10 c.c., +10 c.c. of pyridine | 10·4 | 2772·5 | 87·4 | 20 | 200 | 3% in cathode |
| 3 | 0·017M-Cobalt complex, 10 c.c., +10 c.c. of pyridine | 0·0 | 442·6 | 2·1 | 20 | 200 | 0·5% passing to anode |
| 2 | 0·017M-Cobalt acetate, 20 c.c., +10 c.c. of pyridine | 0·0 | 2701·5 | 76·1 | 90 | 10 | 2·8% in cathode |
| 1 | 0·017M-Complex, 10 c.c., +10 c.c. of pyridine | No activity detected | Not counted | No activity detected | 90 | 10 | — |

*Labelled Complex.*—Bis(salicylideneaniline)cobalt containing $^{60}Co$ was synthesised by the method outlined in Part I for the inactive complex. In this case a quantity of the stock radioactive $^{60}Co$ solution, also prepared by the method outlined in Part I, was added to the cobalt acetate solution used in the preparation so that the crystalline material obtained was labelled in a simple manner. Solutions were prepared by dissolving the correct weight of complex in the appropriate solvent.

*Spectra.*—The spectra were taken with the aid of a Hilger Uvispek spectrophotometer with 1-cm. quartz cells.

*Conductivity Measurements.*—The cell used was similar in design to that used by Pennycuick (*J.*, 1927, 2600) and was kept in a thermostat at 25°. The cell constant was 0·0085. The

solutions used were prepared by diluting 4 c.c. of a 0·1063M-solution of bis(salicylideneaniline)-cobalt in pyridine to 50 c.c. with further pyridine, sufficient water being added to give the appropriate percentage of water. Pyridine–water mixtures were prepared in the same way. The solutions were kept in the bath until temperature equilibrium had been reached, then the resistance was measured with a Pye and Sons manganin-coil resistance bridge, using an A.W.A. oscillator with variable signal strength, and a pair of headphones. With this equipment it was not possible to obtain greater than 2- or 3-figure accuracy when measuring resistances. Conductivity water (0·9 gemmho) was used whenever necessary.

*Solvents.*—All solvents were of reagent grade, purified before use by appropriate procedures. British absolute alcohol " for spectroscopy " was used after refluxing with potassium hydroxide and distillation for the absorption spectra determinations.

JOHNSON CHEMICAL LABORATORIES, UNIVERSITY OF ADELAIDE. [*Received, February 25th*, 1952.]

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## 599. *Properties of Ion-exchange Resins in Relation to Their Structure. Part IV.* Swelling and Shrinkage of Sulphonated Polystyrenes of Different Cross-linking.*

By K. W. PEPPER, D. REICHENBERG, and D. K. HALE.

Two methods are described for determining the weight-swelling (g. of water absorbed per g. of dry resin) of resins in bead form. In the first, a sample of swollen resin is centrifuged at 400 **g** for 30 minutes and then weighed, a correction being applied for residual water retained between the beads after centrifugation; after drying, the sample is re-weighed. The second method involves measurement of the increase in concentration which occurs when dry resin (in the hydrogen form) is added to a dilute solution of the acidic dye, Chlorazol Sky Blue FF. The results obtained by the two methods are in good agreement.

It is shown from pycnometric measurements alone that the hydrogen, sodium, and mixed forms of all the resins obey the relationship, $(V_M)_H = (W_M)_H + 0{\cdot}63$, where $(V_M)_H$ is the swollen volume and $(W_M)_H$ is the weight of water absorbed by an amount of the M-form of the resin equivalent to 1 g. of dry hydrogen form. This relationship enables volume changes to be calculated directly from weight-swelling values.

The effect on weight-swelling has been investigated for changes in the degree of cross-linking of the resin, in the cationic composition of the resin, and in the concentration of ambient hydrochloric acid solutions. The absorption of hydrochloric acid by resins in the hydrogen form has been measured.

ION-EXCHANGE resins are hygroscopic, swelling gels which undergo changes in volume in consequence of changes in environmental conditions, *e.g.*, the relative humidity of the atmosphere or the concentration of an ambient solution. Volume changes may also result from changes in the ionic composition of a resin. These effects are of importance from both practical and theoretical view-points. For example, in column applications a high exchange-capacity per unit volume of swollen resin is required; hence a resin which exhibits high swelling in water is at a disadvantage. Again, swelling and shrinkage effects may disturb the uniformity of packing of a bed of resin and thus give rise to unsatisfactory " fronts " in chromatographic separations. With ion-exchange resins derived from cross-linked copolymers of the styrene–divinylbenzene type, the swelling in water depends on the degree of cross-linking and on the nature and concentration of acidic or basic groups present as substituents. For monosulphonic acids of cross-linked polystyrenes, the swelling decreases with increasing cross-linking (Pepper, *J. Appl. Chem.*, 1951, **1**, 124). Thus, the swelling in water provides a useful criterion of the degree of cross-linking of the polymeric framework, a factor which has been shown to influence certain important ion-exchange

* Part III, *J. Amer. Chem. Soc.*, in the press. Part II, *J.*, 1951, 493.

properties, *e.g.*, the relative affinity coefficients (Reichenberg, Pepper, and McCauley, *J.*, 1951, 493), the rates of exchange (Reichenberg, in the press), and the effective exchange-capacity for large organic ions (Kunin and Myers, *Discuss. Faraday Soc.*, 1949, **7**, 114). The importance of swelling phenomena has been emphasised also in the more recent theoretical treatments of ion-exchange equilibria. In particular, Gregor (*J. Amer. Chem. Soc.*, 1951, **73**, 642) has suggested a direct relationship between the relative affinity coefficient of two ions for a resin, the swollen resin volume, and the partial molal volumes of the hydrated ions within the resin.

For many purposes, information on the volume changes experienced by a resin is of greater interest than the corresponding data relating to changes in weight. The direct determination of the volume-swelling of particulate materials in liquids presents some difficulty but satisfactory results have been obtained by indirect methods. The true volume of a resin saturated in water may be determined pycnometrically provided the weight of water absorbed by the resin is known. Similarly, if the corresponding data are available, the true volume of the resin in contact with aqueous solutions may also be determined. For the measurement of the amount of water absorbed by a resin (the " weight-swelling ") a centrifuge method has proved convenient. Independent confirmation has been obtained in some cases by estimating the increase in concentration which occurred when dry resin (in the hydrogen form) was added to a dilute solution of an acidic dyestuff.

Centrifuge methods have been widely used for determining the weight-swelling of textile fibres (Preston, *J. Text. Inst.*, 1949, **40**, P674). In principle, a sample of material of known dry weight is saturated with water and then re-weighed after being centrifuged to remove excess of liquid. The chief difficulty encountered is that centrifuging—at least at moderate speeds—fails to remove all the water held by capillary forces *external* to the material. In this work, the amount of residual water was estimated from control experiments with surface-sulphonated resins which swelled to a negligible extent.

A similar approach to the determination of the swelling of resins has been described by Gregor, Held, and Bellin (*Analyt. Chem.*, 1951, **23**, 620), who, however, made no correction for the residual water.

## Experimental

*Materials.*—Sulphonated polystyrenes of different cross-linking were prepared in bead form by methods previously described (Pepper, *loc. cit.*). The cross-linking agent was divinylbenzene, which was supplied commercially in the form of a solution in ethylstyrene. During the course of the work, two series of resins, differing with respect to the divinylbenzene solution employed, were prepared. The first series was made from a divinylbenzene solution supplied by Monsanto Chemicals Ltd. containing approximately 33% of divinylbenzene; these resins were used for the studies of relative affinity coefficients previously reported (Reichenberg *et al.*, *loc. cit.*). The second series was made from a solution containing approximately 50% of divinylbenzene, obtained from the Dow Chemical Co. The two series were matched on the basis of the swelling of the unsulphonated copolymers in toluene. The divinylbenzene contents of the monomer mixtures required to give copolymers of equal swelling were found to be in fair agreement. After sulphonation, corresponding members of the two series exhibited the same swelling in water within experimental error.

Surface-sulphonated resins were prepared by treating cross-linked polystyrene beads with sulphur trioxide vapour at 0° for 20—25 minutes. The exchange-capacity of the products decreased with increasing particle diameter; thus, for fractions 100—200 μ and 300—400 μ in diameter, the capacities were 0·03 and 0·01 mg.-equiv. per g. (compared with 5·2 mg.-equiv. per g. for fully sulphonated resin). The surface-sulphonated resins were readily wetted with water and clearly present a hydrophilic surface comparable with that of the fully sulphonated materials.

*Determination of Weight-swelling.—Centrifuge method.* A sample of resin (approx. 1 g. wet weight) was introduced into a glass filter-tube (Fig. 1) fitted with a sintered-glass disc of porosity 2, 3, or 4. The tube and resin were immersed in de-ionised water at 25° for about 1 hour and then placed in a glass centrifuge tube (15 ml.) which was closed with a rubber cap in order to prevent evaporation of water during centrifuging. Since the rubber cap became deformed during centrifuging and tended to seal the filter-tube, two small holes were blown in the filter-tube to equalise pressure inside and outside. The tube was centrifuged at 400 **g** for 30 minutes,

the temperature being maintained at 25° by running the laboratory centrifuge (with the lid open) in an air-thermostat. The filter-tube was weighed and re-immersed in water, and the operation repeated. The filter-tube was finally heated at 110° overnight and then dried to constant weight by heating it at 110° at a pressure of 1 mm. Hg with a trap containing phosphoric oxide in the vacuum line. Previously, the weight of the empty filter-tube had been obtained after drying it at 110° and after centrifugation with water alone. These data give the dry weight of the sample of resin and also the wet weight, uncorrected for residual water.

The wet weight of resin was reproducible to $\pm 2$ mg. for periods of centrifuging from 30 minutes to 6 hours; losses due to evaporation were completely prevented by the rubber caps. Within the same limits, the wet weight of all the resins examined was constant for centrifuge speeds varying from 1500 to 2300 r.p.m., corresponding to 225 to 530 **g**. Some deswelling with increase in the centrifugal field might be expected with very lightly cross-linked resins. Calculation indicates, however, that the effect is insignificant at the speeds used even with a resin of 2% nominal divinylbenzene content, which was the most lightly cross-linked resin examined. It may be noted, however, that Preston (*J. Text. Inst.*, 1951, **42**, T79) has observed that the amount of residual water decreases linearly with the reciprocal of the centrifugal field at much higher speeds (1000—5000 **g**).

The amount of residual water held externally to the beads of resin after centrifuging was estimated from control experiments with surface-sulphonated polystyrene. A filter-tube was

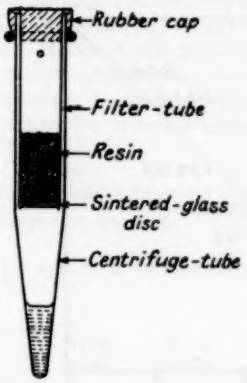



FIG. 1. *Centrifuge-tube assembly.*

filled with water, centrifuged under the standard conditions described above, and weighed. A known weight of surface-sulphonated resin was added to the tube, which was then immersed in water, centrifuged, and re-weighed. The amount of residual water was found to be proportional to the weight of resin taken. The experiment was repeated with surface-sulphonated resins of different particle diameter. Within the range 100—400 $\mu$, no trend with size was observed, a mean value of $50 \pm 5$ mg. of water per g. of resin being obtained. This value corresponds to 53 mg. per ml. of resin, the density of the resin being 1·06 g. per ml.

An upper limit for the amount of residual water was obtained, independently, from experiments on the absorption of hydrochloric acid by a very highly cross-linked resin (see below). Experiments with samples of different particle diameter again showed no trend within the range 150—400 $\mu$ and gave a mean value of $58 \pm 4$ mg. per ml. of resin. This is in reasonable agreement with the previous value obtained gravimetrically, which was therefore applied as a correction to the weight of wet resin. This correction, which is about 4% of the wet weight, is known to an accuracy of $\pm 10\%$; the error from this source may therefore be $\pm 0{\cdot}4\%$ of the weight of wet resin. The overall accuracy of the wet weight per g. of dry resin is 1—2%. For clarity the results are expressed as g. of water absorbed per g. of dry resin.

The centrifuge method was used for the determination of the weight-swelling of resins of different cationic composition. The resin sample in the filter-tube was converted into a known cationic composition by treatment with an appropriate " conditioning " solution (Reichenberg *et al.*, *loc. cit.*). The weight-swelling of the conditioned resin in contact with the solution was obtained by centrifuging it immediately; alternatively, the sample was washed with water and then centrifuged, thus giving the weight-swelling of the conditioned resin in contact with water.

The weight of water absorbed per g. of dry conditioned resin is obtained directly; knowing the cationic composition of the resin and its specific exchange-capacity, the weight of water absorbed by an amount of conditioned resin equivalent to 1 g. of the dry hydrogen form may be calculated.

*" Negative absorption " method.* Weight-swellings were also determined by measuring the increase in concentration of a dilute solution of a reference solute on addition of the dry resin. The acidic dye Chlorazol Sky Blue FF was selected as reference solute since it may be determined colorimetrically in very dilute solution, and the anion diffuses very slowly and to an extremely small extent into cation-exchange resins (Kressman and Kitchener, *Discuss. Faraday Soc.*, 1949, **7**, 90). A 0·05% solution of the pure dye (Colour Index No. 518) was prepared. The dye had been purified by precipitating the commercial product with sodium acetate and washing the precipitate with boiling ethyl alcohol. This procedure had been repeated three times and the dye dried at 100° overnight.* When this solution was passed through a column containing sulphonated cross-linked polystyrene (hydrogen form), there was no indication of any absorption of dye by the resin. The solution of the dye in the free acid form was then diluted to a concentration of 0·01% (wt./vol.). An accurately weighed sample (0·5—2·0 g.) of the sulphonated cross-linked polystyrene (hydrogen form) which had been dried at 110° as described above was treated with 5 ml. of the 0·01% solution. After 2 hours with occasional shaking, the solution

FIG. 2. *Comparison of the results obtained by two methods of determination of weight-swelling.*

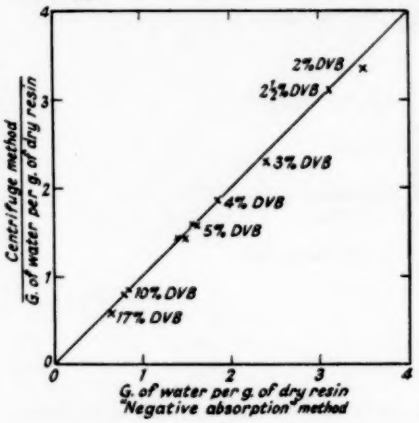
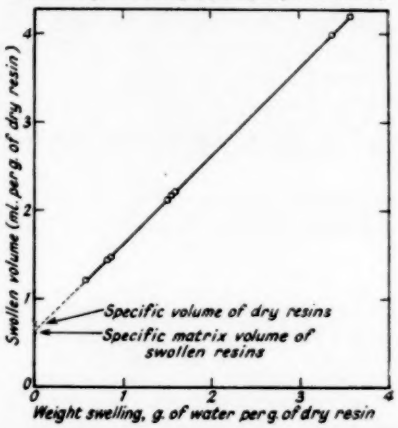



FIG. 3. *Relationship between swollen volume and weight-swelling (hydrogen-form resins).*


Swollen volume (ml. per g. of dry resin)
Specific volume of dry resins
Specific matrix volume of swollen resins
Weight swelling, g. of water per g. of dry resin

was filtered off from the resin, and the concentration of the dye was measured colorimetrically, a Hilger Spekker absorptiometer being used. The filtrate sample was diluted with water and pyridine to give a final pyridine concentration of 25% (v/v) and a dye concentration of about 0·001% which was found suitable for an accurate determination.

Variation in the concentration of dye solution from 0·002% to 0·05% caused no significant difference in the values obtained for weight-swelling.

*Comparison of Results Obtained by Centrifuge and " Negative Absorption " Methods.*—The weight-swellings of a number of samples of sulphonated polystyrenes of different degrees of cross-linking and particle-size were determined by the two methods, which, as shown by Fig. 2, gave results in good agreement. Also, for a given degree of cross-linking, the values of weight-swellings obtained were independent of particle-size. It may be noted that the results obtained by the " negative absorption " method must represent minimum values for the weight-swelling, since, if the dye is absorbed by the resin or if the swelling of the resin is affected by the concentration of the dye in solution, a low result would be given.

*Determination of Volume-swelling.*—The weight of water displaced by a sample of swollen resin of known cationic composition was determined at 25·00° ± 0·02°, a 5-ml. density bottle being used. The dry weight of the sample was obtained by transferring the resin quantitatively to a small filter-tube and then heating it at 110° *in vacuo* to constant weight as described pre-

* The pure Chlorazol Sky Blue FF was kindly supplied by Imperial Chemical Industries Limited, Dyestuffs Division.

viously. The weight of the swollen sample can be calculated from this dry weight and the weight-swelling, and hence the true swollen volume per g. of dry resin can be obtained.

Specific volumes of the dry resins were determined directly, dry *n*-heptane being used as displacement medium.

*Absorption of Hydrochloric Acid by Resins in the Hydrogen Form.*—Resin samples in filter-tubes were conditioned with hydrochloric acid solution of known concentration until equilibrium was reached with respect to the weight-swelling and also the amount of hydrochloric acid absorbed. A 30-fold excess of acid relative to the capacity of the resin sample was normally used at a flow-rate of 100 ml. per hour. After conditioning, the resins were centrifuged and weighed; all the acid was then washed out with de-ionised water, and the washings titrated with standard sodium hydroxide solution. The operation was repeated with hydrochloric acid solutions of different concentrations, and finally the resins were dried to constant weight.

From these measurements, it was possible to calculate both the weight of water and the amount of hydrochloric acid absorbed per g. of dry resin for each acid concentration. Corrections were applied for the amounts of water and acid left in the sintered disc of the filter-tube and also retained between the resin beads after centrifugation.

## Results and Discussion

*Relationship between Weight-swelling and Swollen Volume.*—It was originally intended to measure volume changes indirectly by first determining weight-swellings and then combining these values with pycnometric data. However, the pycnometric data alone indicate that a simple linear relationship between weight-swelling and swollen volume is applicable over a wide range, and hence, within this range, changes in weight-swelling are a reliable measure of volume changes. From the pycnometric measurements *per se* we obtain directly the quantity $A$ given by

$$A = \frac{\text{Weight of swollen resin} - \text{Weight of equal volume of water}}{\text{Weight of dry resin}}$$

The mean values of $A_H$ obtained from pycnometric measurements on the hydrogen forms of different resins are given below:

| Nominal % of divinylbenzene | 2 | 5 | 10 | 15 |
|---|---|---|---|---|
| $A_H$ (Monsanto DVB solution) | 0·373 | 0·373 | 0·376 | — |
| $A_H$ (Dow DVB solution) | 0·378 | 0·373 | 0·373 | 0·377 |

Statistical analysis confirmed that there was no significant variation in the values of $A_H$ either with divinylbenzene content or with source of the divinylbenzene solution; the grand mean of all results was 0·374 (standard deviation 0·004).

It follows from the definition of $A$ above that if $W$ is the weight of water absorbed per g. of dry resin (the weight-swelling) and $V$ is the volume of swollen resin per g. of dry resin, then $A = 1 + W - 0{\cdot}997V$, where 0·997 g. per ml. is the density of water at 25°. Since $A_H$ is a constant (equal to 0·374), $V_H = 1{\cdot}003W_H + 0{\cdot}628$. This equation covers the swollen hydrogen forms of all resins of divinylbenzene content from 2 to 15%. On consideration of the variation in swelling with decrease in cross-linking, it appears that the absorption of each additional gram of water is accompanied by an increase in volume of almost exactly 1 ml., *i.e.*, the partial specific volume of water in the swollen resins is the same as in the free state.

It is of interest to consider how far this concept can be extended to cover the variation in swelling of an individual resin with change in relative humidity. If the linear relationship were valid over the whole moisture isotherm, the specific volume of all the dry resins would be 0·63 ml. per g. The densities of the dry resins (hydrogen form) are in fact the same within experimental error; the observed value of 1·43 ± 0·03 g. per ml., however, corresponds to a specific volume of 0·70 ml. per g., which is 0·07 ml. per g. greater than the previous figure. Hence, when a dry or nearly dry resin is allowed to swell in water, there is a contraction in the total volume of the system and at low swellings the volume must increase by less than 1 ml. for each gram of water absorbed. A similar effect is observed

with cellulosic materials and other swelling gels. The value of 0·63 ml. per g. will be referred to as the *specific matrix volume* of the swollen resins in the hydrogen form, it being understood that this includes the volume of the sulphonic acid groups. These results are illustrated in Fig. 3, where the individual points have been calculated by combining the mean value of $A_H$ for each resin with the value of $W_H$ obtained by the centrifuge method.

The linear relationship is obeyed by different resins possessing weight-swellings ranging from 3·5 to 0·6 g. of water per g. of dry resin; hence for an individual resin, although the linear relationship is not valid over the whole isotherm, it is probably applicable down to weight-swellings of 0·6 g. of water per g. of dry resin or less. For most resins this corresponds with a relative humidity of about 75% (Pepper, *loc. cit.*).

Pycnometric measurements on the sodium forms of different resins gave a constant value for $A_{Na}$ with a mean of 0·432 (standard deviation 0·006). Hence the specific matrix volume of the swollen sodium salts of all the resins is 0·569 ml. per g. This value is referred to 1 g. of dry sodium resin and cannot be compared directly with the previous figure, which relates to 1 g. of dry hydrogen resin. Taking the specific exchange-capacity as 5·2 mg.-

FIG 4.

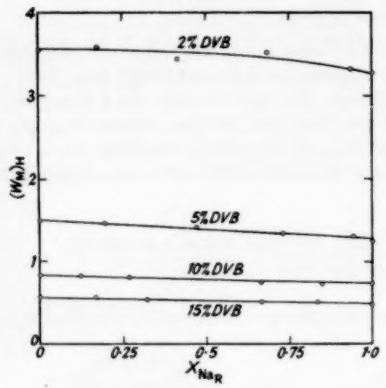
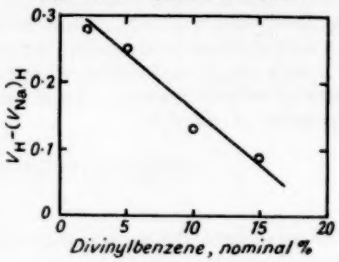



FIG. 5. $V_H - (V_{Na})_H$ *for resins of different divinylbenzene content.*




equiv. per g. of dry hydrogen resin, we calculate that 1 g. of dry hydrogen resin is equivalent to $1{\cdot}11_5$ g. of dry sodium resin. The decrease in capacity (5·4 to 5·0) observed with increase in cross-linking affects this factor by less than 1%. Hence the matrix volume of an amount of swollen sodium resin equivalent to 1 g. of dry hydrogen resin is $0{\cdot}569 \times 1{\cdot}11_5 = 0{\cdot}633$ ml., which differs very slightly from the value of 0·628 ml. obtained on the hydrogen resins. The mixed sodium–hydrogen forms of different resins gave a similar result. Hence, we conclude that the hydrogen, sodium, and mixed forms of all the resins obey the relationship

$$(V_M)_H = (W_M)_H + 0{\cdot}63$$

where $(V_M)_H$ is the swollen volume and $(W_M)_H$ is the weight of water absorbed by an amount of the M-form of the resin equivalent to 1 g. of the dry hydrogen form.

Gregor, Gutoff, and Bregman (*J. Colloid Sci.*, 1951, **6**, 245) have given the swollen weights and swollen volumes of various cationic forms of Dowex-50. This commercial resin is believed to be a sulphonated polystyrene containing about 8% of divinylbenzene. From their data we calculate the following values of $(V_M)_H - (W_M)_H$:

| H | Li | Na | K | Rb | Cs | $NH_4$ | $NEt_4$ |
|---|---|---|---|---|---|---|---|
| 0·644 | 0·634 | 0·656 | 0·700 | 0·729 | 0·745 | 0·738 | 1·325 |

The agreement between their results and our own for the sodium form is not good; it appears, however, that the above relationship may be valid for the mixed forms of the resin involving hydrogen, lithium, and sodium, but not for the other univalent cations.

It may be noted that our results were obtained on resins possessing approximately the

same specific exchange-capacity. If the capacity were less than that corresponding to monosulphonation, the linear relationship might still hold but the matrix volume would be greater than 0·63 ml.

*Effect of Degree of Cross-linking and Cationic Composition of the Resins on Swelling.*— Weight-swellings of the hydrogen forms of resins prepared from the Dow divinylbenzene solution are shown in the following table; similar data for resins prepared from the Monsanto divinylbenzene solution have been reported (Pepper, *loc. cit.*, Fig. 7).

| Nominal % of divinylbenzene ..... | 2 | 2½ | 4½ | 5 | 7½ | 10 | 15 | 25 |
|---|---|---|---|---|---|---|---|---|
| Weight-swelling ..... | 3·55 | 3·10 | 1·78 | 1·50 | 1·10 | 0·82 | 0·58 | 0·38 |

The above swelling values are means obtained from a number of preparations at each nominal divinylbenzene content; individual preparations showed some variation from these means (cf. next table). It will be seen that weight-swelling is a sensitive criterion of the degree of cross-linking up to a nominal divinylbenzene content of about 10% but that the sensitivity of this test falls off at higher divinylbenzene contents. However, in this latter range, the relative affinity coefficients of the resins for sodium and hydrogen are markedly

FIG. 6. *Absorption of hydrochloric acid.*

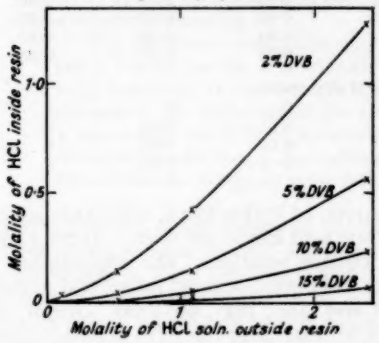
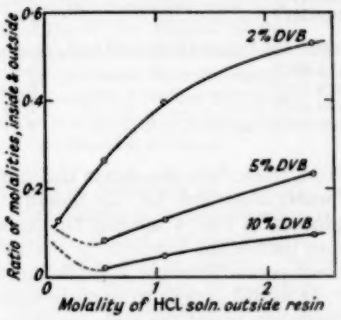



FIG. 7. *Ratio of molalities inside and outside resin as a function of molality outside.*




affected by divinylbenzene content and provide a satisfactory criterion (Reichenberg *et al.*, *loc. cit.*).

Swelling changes resulting from variation in the cationic composition of the resin have been determined for the sodium–hydrogen system. In Fig. 4, $(W_H)_M$, *i.e.*, the weight of water absorbed by an amount of the mixed sodium–hydrogen resin equivalent to 1 g. of dry hydrogen form, is plotted against the equivalent fraction of sodium on the resin, $X_{Na_R}$. The data presented refer to resins in contact with water. Somewhat lower values were obtained in contact with the 0·1N-conditioning solutions, but this effect was appreciable only with the 2% divinylbenzene resin where the difference was 0·2 g. of water per g. of dry resin.

It will be seen from Fig. 4 that more water is absorbed by the hydrogen form of the resins than by an equivalent amount of the sodium form. Moreover, the swollen volume of the hydrogen form is greater than that of the sodium form, which is in accord with other evidence that the hydrated hydrogen ion is larger than the hydrated sodium ion (Boyd, Schubert, and Adamson, *J. Amer. Chem. Soc.*, 1947, **69**, 2818; Kressman and Kitchener, *J.*, 1949, 1190; Gregor, Gutoff, and Bregman, *loc. cit.*). The difference between the swollen volumes of the two forms, *i.e.*, $V_H - (V_{Na})_H$, decreases with increasing cross-linking, tending to a value of 0·3 ml. per g. of dry resin at low divinylbenzene content and to zero at high divinylbenzene content (Fig. 5). This effect is consistent with Gregor's treatment of the swelling of ion-exchange resins (*J. Amer. Chem. Soc.*, 1951, **73**, 642). He suggests that some of the water in a swollen resin is bound fairly strongly to the various ions whilst the rest is " free " water. When one cation is replaced by a larger cation, the resin expands but

the elastic forces of the polymer network (which tend to resist any increase in total volume) are increased. As a result, " free " water is squeezed out. Hence the net increase in volume is less than the difference between the volumes of the two hydrated cations. The elastic forces, and hence the amount of " free " water squeezed out, increase with increasing cross-linking.

*Swelling in Hydrochloric Acid Solutions and Absorption of Hydrochloric Acid.*—The amounts of water absorbed by resins of different divinylbenzene content (hydrogen form) in contact with hydrochloric acid solutions of varying concentration, and the corresponding amounts of hydrochloric acid taken up by the resins, are given below. In both cases, the greatest effect is observed with the resin of lowest cross-linking. Absorption of hydrochloric acid by the 25%-divinylbenzene resin was negligible; the amount of acid washed out was equal within experimental error to the correction for residual solution retained between the beads after centrifugation.

| | Nominal % of divinylbenzene | | | | |
|---|---|---|---|---|---|
| | 2 | 5 | 10 | 15 | 25 |
| Weight-swelling (g. of water per g. of dry resin): | | | | | |
| $H_2O$ | 3·26 | 1·57 | 0·88 | 0·60 | 0·36 |
| 0·11N-HCl | 3·14 | 1·52 | 0·88 | 0·60 | 0·36 |
| 0·52N-HCl | 2·80 | 1·48 | 0·86 | 0·60 | 0·35 |
| 1·05N-HCl | 2·41 | 1·38 | 0·84 | 0·59 | 0·35 |
| 2·30N-HCl | 1·81 | 1·24 | 0·78 | 0·57 | 0·35 |
| Absorption of hydrochloric acid (mg.-equiv. of HCl per g. of dry resin): | | | | | |
| 0·11N-HCl | 0·04 | 0·02 | 0 | 0 | 0 |
| 0·52N-HCl | 0·39 | 0·06 | 0·01 | 0·01 | 0 |
| 1·05N-HCl | 1·03 | 0·19 | 0·04 | 0·02 | 0 |
| 2·30N-HCl | 2·31 | 0·70 | 0·18 | 0·04 | 0 |

From these data we obtain the number of mg.-equiv. of hydrochloric acid taken up per g. of water absorbed, *i.e.*, the molality of hydrochloric acid inside the resin. These values are plotted in Fig. 6 against the molality of the outside solution. The applicability of Donnan membrane concepts to ion-exchange resins has been discussed by several authors in recent years (Bauman and Eichhorn, *J. Amer. Chem. Soc.*, 1947, **69**, 2830; Gregor, *loc. cit.*). It would appear from the simpler treatments, at least, that the ratio of hydrochloric acid molality inside the resin to that outside should tend to zero at zero external concentration and to unity at high external concentrations. Our data for the 2%-divinylbenzene resin, which are the most reliable, are consistent with this concept (Fig. 7). With the other resins, there appears to be a minimum at low external concentrations; however, a small experimental error would account for the effect. Where little reliance can be placed on the shape of the curves in Fig. 7, broken lines have been used.

The work described above has been carried out as part of the research programme of the Chemical Research Laboratory, and this paper is published by permission of the Director of the Laboratory.

The authors acknowledge the assistance rendered by Mr. L. R. Sud, M.Sc., Mr. H. M. Paisley, Mr. W. F. Wall, and Mr. M. A. Young, B.Sc., who performed the experimental work.

CHEMICAL RESEARCH LABORATORY, TEDDINGTON, MIDDLESEX. [*Received, March 12th*, 1952.]

## 600. *The Effect of Solvent on the Ultra-violet Absorption Spectra of Aromatic Hydrocarbons with Special Reference to the Mechanism of Salting-out. Part I. Alcoholic Lithium Chloride Solutions.*

By E. F. G. Herington and W. Kynaston.

The failure of the majority of published mechanisms of salting-out to account for all the experimental observations is discussed briefly. The possibility of obtaining some information on the environment of non-electrolytes in salt solutions from the study of ultra-violet spectra is considered.

The spectra of benzene, *m*-xylene, naphthalene, anthracene, and chrysene dissolved in alcoholic lithium chloride solutions are compared with the spectra of the same aromatic hydrocarbons in alcohol. Comparison is also made of the spectra of these aromatic hydrocarbons dissolved in alcohol with their spectra when dissolved in *cyclo*hexane, and of the spectrum of benzene dissolved in *cyclo*hexane with its spectrum in *n*-hexane. The effect of lithium chloride on the spectrum of benzyl alcohol in the absence of a diluent is recorded.

The observed changes in spectra are shown to be consistent with the salt's having caused a modification of the structure of the alcohol in the neighbourhood of the non-electrolyte. The spectra also indicate that the changes in the structure of the alcohol extend some distance from the ions: a conclusion which is in agreement with published entropy values for ions in alcoholic solutions. Any adequate theory of the mechanism of salting-out from alcoholic solutions must take cognisance of this structural factor.

The simplest and oldest theory of the effect of a salt on the solubility of a weakly polar material in an ionising solvent assumes that the salt immobilizes a certain fraction of the solvent which is then no longer available for dissolution of the non-electrolyte. The unbound solvent is considered to be in the same state as when salt is absent. Solvation numbers can be calculated in this manner by measuring the reduction in solubility of a non-electrolyte produced by dissolving a salt. For example, Kosakewitsch (*Z. physikal. Chem.*, 1929, *A*, **143**, 216) derived a solvation number of 6·2 for lithium chloride in a N-lithium chloride alcoholic solution, using carbon dioxide as the non-electrolyte. This simple theory requires that the fraction of non-electrolyte salted out by a given concentration of a given electrolyte from a given solvent should be independent of the actual non-electrolyte employed, but because this is usually not found to be true additional postulates have to be made (see Bockris, *Quart. Reviews*, 1949, **3**, 177). For instance, Eucken and Hertzberg (*Z. physikal. Chem.*, 1950, **195**, 1) studied the salting-out effect of sodium, potassium, lithium, and magnesium chlorides on argon, krypton, xenon, oxygen, methane, and ethane from aqueous solutions, and calculated hydration numbers which were approximately independent of the non-electrolyte by allowing for the depolymerisation of the water by the salt and for the hydration of the non-electrolyte. However, this treatment requires additional justification because recent work on the self-diffusion of water indicates that no definite species of associated water molecules are present in liquid water (Wang, *J. Amer. Chem. Soc.*, 1951, **73**, 510, 4181).

Mathematical theories of salting-out from very dilute electrolyte solutions have been advanced by Debye and McAulay (*Physikal. Z.*, 1925, **26**, 22), Debye (*Z. physikal. Chem.*, 1927, **130**, 56), Butler (*J. Phys. Chem.*, 1929, **33**, 1015), Gross *et al.* (*Monatsh.*, 1929, **53**, 445; 1930, **55**, 287), and Altshuller and Everson (*J. Phys. Chem.*, 1951, **55**, 1368). These authors do not consider the molecular structure of the solvent but utilize bulk properties of the system such as the dielectric constant to calculate the changes in concentration resulting from the tendency of the more polarisable molecules to collect round the ions. Some general qualitative agreement is often found between these treatments and experiment (see Randall and Failey, *Chem. Reviews*, 1927, **4**, 271; Gross, *ibid.*, 1933, **13**, 91;

Heal, *Canadian J. Chem.*, 1951, **29**, 563), but these theories do not explain all the observations, and in particular they are inconsistent with the occurrence of "salting-in" and "salting-out" of one non-electrolyte in a given solvent by two different salts (see, *e.g.*, Gross, *loc. cit.*; Harned and Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publ. Corp., 1950, p. 400). Factors other than the electrostatic forces must therefore also operate, as Debye himself pointed out (*loc. cit.*, 1927).

Recently, there have been attempts to explain salt effects as a structural phenomenon, and for this purpose the entropy change accompanying the transference of nitrous oxide (or oxygen) from an aqueous alkali halide solution to pure water has been computed (Frank and Evans, *J. Chem. Physics*, 1945, **13**, 507). This entropy change was found to be negative for nitrous oxide in lithium, potassium, and rubidium chloride solutions and in potassium bromide and iodide solutions. Such values can only be explained on Debye and McAulay's theory if additional *ad hoc* assumptions are made on the temperature-dependence of the dielectric constant and on the ionic salting-out radii. A tentative explanation of these observations was advanced in terms of the orientation of the solvent structure by the ions and by the non-electrolyte.

Clearly, any measurements which would give an indication of the conditions existing in the vicinity of the non-electrolyte molecules in a salt solution might contribute to a better understanding of the mechanism of salting-out. A number of studies of the effects of electrolytes on the spectra of coloured materials and on indicators has been published, but because the compounds employed were acids, bases, or substances with large complex molecules the interpretation of the results has been complicated by possible changes in dissociation and aggregation of the molecules containing the chromophore. To avoid these complications the effects of lithium chloride on the ultra-violet spectra of various aromatic hydrocarbons dissolved in ethyl alcohol have now been studied, and the results are reported in the present paper; in Part II (following paper) the effects of salts on the spectra of benzene and naphthalene dissolved in water are examined. For the present purpose ethyl alcohol was chosen as the solvent because it is transparent to ultra-violet light, and lithium chloride was chosen as the salt because it is a strong electrolyte in alcohol (Connell, Hamilton, and Butler, *Proc. Roy. Soc.*, 1934, *A*, **147**, 418). There is some evidence that hydrogen bonding takes place between alcohols and aromatic hydrocarbons in solution (Searles and Tamres, *J. Amer. Chem. Soc.*, 1951, **73**, 3704) but the occurrence of such interaction does not invalidate the conclusions of the present paper.

The changes produced by lithium chloride in the spectra of an alcoholic solution of an aromatic hydrocarbon were measured directly by the four-cell technique described below. The results for benzene, *m*-xylene, naphthalene, anthracene, and chrysene are shown in Fig. 1, where the differences ($\Delta\varepsilon$) between the molar extinction coefficients of the solution under study and of the alcoholic solution are plotted against wave-length expressed in mμ. In this paper all values of $\Delta\varepsilon$ are measured with respect to the corresponding alcoholic solution. Table I summarises the maximum errors to be expected in $\Delta\varepsilon$ for solutions of these compounds in N-lithium chloride and in *cyclo*hexane. These maximum errors were assessed from comparisons of pairs of identical solutions.

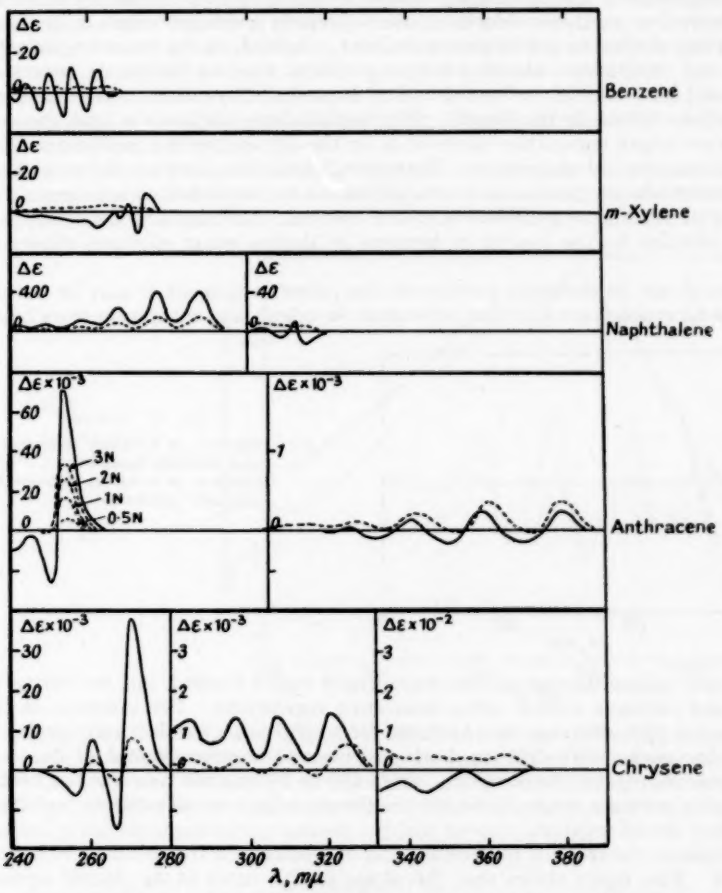
TABLE 1. *Maximum error to be expected in* $\Delta\varepsilon$.

| Hydrocarbon | | $\pm\Delta\varepsilon$ N-Lithium chloride | $\pm\Delta\varepsilon$ *cyclo*-Hexane |
|---|---|---|---|
| Benzene | | 0·5 | 0·5 |
| *m*-Xylene | | 1 | 2 |
| Naphthalene | 240—290 mμ | 10 | 15 |
| | 290—320 mμ | 0·5 | 1 |
| Anthracene | 240—260 mμ | 1100 | 1100 |
| | 300—390 mμ | 40 | 40 |
| Chrysene | 250—280 mμ | 500 | 500 |
| | 280—330 mμ | 50 | 50 |
| | 330—370 mμ | 10 | 10 |

The data in Fig. 1, taken in conjunction with the values in Table 1, show that 1N-lithium chloride affects the spectra of all these aromatic hydrocarbons dissolved in alcohol to an extent exceeding the experimental error of measurement. This observation is not consistent with the simple hypothesis that the only action of salt is to immobilize a portion

of the solvent. Fig. 1 also shows the values of $\Delta\varepsilon$ obtained with anthracene in the wave-length region 250—260 mμ for 0·5, 1·0, 2·0, and 3·0N-lithium chloride solutions. The values of $\Delta\varepsilon$ increase numerically with increase in salt concentration, but there is no simple relationship between the numerical values of $\Delta\varepsilon$ at any fixed wave-length and the concentration of the electrolyte.

FIG. 1.




——— *Difference in ε values* (Δε) *between* cyclo*hexane and alcoholic solutions.*
– – – – *Difference in ε values* (Δε) *between* 1N-LiCl *and alcoholic solutions.*

The observed changes in the spectra (Δε) produced by lithium chloride might be caused by direct electrostatic interaction of the ions with the chromophores or by a modification of the interaction of the solvent molecules with the absorbing aromatic nuclei. Both of these mechanisms may operate, but if the aromatic molecules tend to occupy regions of the solution far removed from the ions, as the Debye theory requires, then the hydrocarbon molecules will be shielded from direct electrical effects by alcohol molecules and the observed values of $\Delta\varepsilon$ will probably arise mainly from changes in solvent–chromophore interaction. A 50-fold increase in the concentration of anthracene and a decrease in the path length in the same ratio for a 1N-lithium chloride solution gave a curve of $\Delta\varepsilon$ in the region 240—260 mμ which was almost identical with that for the lower anthracene concentration

in a similar salt solution. This observation is compatible with the majority of the aromatic molecules in these solutions occupying parts of the solution remote from the ions. The following observations support the thesis that the observed values of $\Delta\varepsilon$ for salt solutions arise mainly from a change in solvent–chromophore interaction.

If the alterations in the spectra caused by lithium chloride are a result of changes in the structure of the alcohol, then it might be expected that the observed $\Delta\varepsilon$ values would be of similar magnitude to the $\Delta\varepsilon$ values produced by changing to a completely different solvent. Fig. 1 shows that N-lithium chloride in alcohol results in smaller values of $\Delta\varepsilon$ than does the change from alcohol to *cyclo*hexane as solvent. Indeed, in the wave-length region 250—270 mμ the 1N-lithium chloride solution produces maxima in the $\Delta\varepsilon$ curves which are numerically from one-fifth to one-third of those produced by *cyclo*hexane for all the aromatic hydrocarbons shown in the figures. For naphthalene, anthracene, and chrysene in the higher wave-length regions the values of $\Delta\varepsilon$ for the salt and for the *cyclo*hexane solutions are of a similar numerical magnitude. The general parallelism between the magnitudes of the $\Delta\varepsilon$ values for lithium chloride and for *cyclo*hexane is compatible with a common origin for the effect of salt and of *cyclo*hexane on the spectra. Additional evidence supporting this view is afforded by the spectra of benzene in alcohol–water mixtures shown in Fig. 3, Part II.

Although not immediately germane to the present argument, it may be noted that the $\Delta\varepsilon$ curves for *cyclo*hexane solutions of benzene, *m*-xylene, and anthracene show both positive

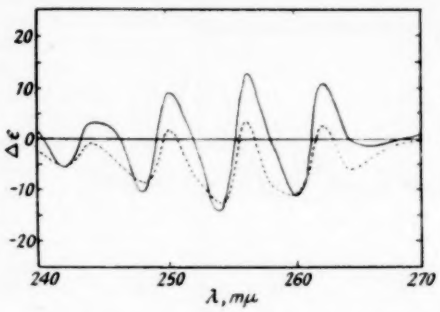



FIG. 2. *Benzene.*
——— *Difference in ε values between* cyclo*hexane and alcoholic solutions.*
- - - - *Difference in ε values between hexane and alcoholic solutions.*

and negative values throughout the wave-length region studied, but the curves for naphthalene and chrysene exhibit some interesting regularities. For instance, in the wave-length region 240—290 mμ, the $\Delta\varepsilon$ curve for naphthalene exhibits only positive values, while in the region 290—320 mμ both positive and negative values of $\Delta\varepsilon$ occur. The chrysene curves exhibit three regions: from 250 to 270 mμ the $\Delta\varepsilon$ curve has both positive and negative portions, from 280 to 330 mμ the $\Delta\varepsilon$ values are all positive, and from 330 to 370 mμ they are all negative.

Fig. 2 allows the effect of *cyclo*hexane and of *n*-hexane on the spectrum of benzene to be compared. This figure shows that the shape of the curve of $\Delta\varepsilon$ plotted against wave-length is very sensitive to the structure of the solvent. This observation indicates that, although for some hydrocarbons the values of $\Delta\varepsilon$ at a certain wave-length for the lithium chloride and for the *cyclo*hexane solutions may be of opposite sign (*e.g.*, chrysene in region 330—370 mμ), yet this behaviour is not incompatible with the effect of the salt being due to a modification in the structure of the solvent.

If the spectral differences produced by lithium chloride in ethyl-alcoholic solutions were due to a direct electrical interaction of the ions with the aromatic molecules, then it would be expected that solution of this salt in benzyl alcohol (with no additional solvent) would cause a spectral change in benzyl alcohol of the same type as is shown in Fig. 1 but of larger magnitude. Curves of $\Delta\varepsilon$ plotted against wave-length could not be obtained directly for benzyl alcohol because of the difficulty of manipulating capillary-thickness absorption cells, but the optical densities of a solution of 1N-lithium chloride in benzyl alcohol and of benzyl alcohol alone were measured. These spectra are shown in Fig. 3, where the absorp-

tion peaks at 259 mμ have been adjusted arbitrarily to equality by multiplying all the measured optical densities for one of the curves by a constant factor. A very small difference is found between the curves for benzene in 1N-alcoholic lithium chloride and for benzene in ethyl alcohol if these spectra are plotted in the same manner as in Fig. 3, although the salt curve does lie slightly above that for the ethyl-alcoholic solutions at the minima when these curves are adjusted to equality at 255 mμ. The effect of 1N-lithium chloride on the spectrum of benzyl alcohol is thus much greater than on benzene in ethyl alcohol, but is different in type, since the benzyl alcohol curve shows more structure in the presence of salt and lies below the benzyl alcohol curve, while the curve for benzene shows less structure in the presence of salt and lies above the curve for benzene in alcohol. The spectral changes produced by the salt and illlustrated in Fig. 3 may be a result of the electrostatic field of the ions, but the spectral shifts recorded in Fig. 1 for the ethyl-alcoholic salt solutions probably have a different origin.

These observations on the spectra of ethyl-alcoholic lithium chloride solutions appear to afford direct experimental evidence of a modification in the structure of the alcohol by the ions even at some distance from the ion. This conclusion supports Eley and Pepper's

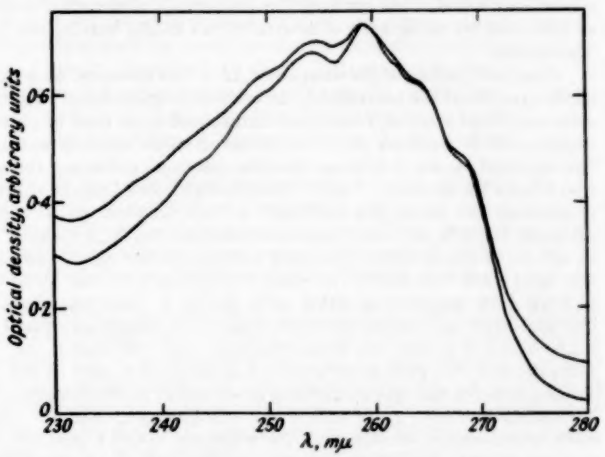



FIG. 3.

*Upper curve : Benzyl alcohol.*
*Lower curve : Benzyl alcohol* + LiCl.

tentative suggestion (*Trans. Faraday Soc.*, 1941, **37**, 581) that the shell of dipoles orientated by ions in methyl-alcoholic solutions causes the re-orientation of the solvent molecules in the bulk of the solution. Ions in ethyl alcohol would be expected to behave similarly. Obviously, any complete theory of salting-out from alcoholic solutions must take cognisance of the change in the structure of the solvent induced by the salt.

## EXPERIMENTAL

*Preparation of Compounds.*—The benzene and *m*-xylene were standard specimens prepared at the Chemical Research Laboratory and were 99·99 ± 0·005 and 99·97 ± 0·02 mol. % pure, respectively, as established by freezing-point determinations. The naphthalene and anthracene were kindly supplied by the North Thames Gas Board : the former had a purity of greater than 99·99 mol. %, and the latter was a synthetic sample. The chrysene was a pure commercial sample which was recrystallised from alcohol. The benzyl alcohol was a redistilled commercial product.

The lithium chloride was purified and dried by Shaw and Butler's method (*Proc. Roy. Soc.*, 1930, *A*, **129**, 519). Their procedure was modified slightly in that the salt was washed with purified dry ether after crystallisation from alcohol and before drying in a stream of dry hydrogen chloride.

Commercial ethyl alcohol was used as a solvent without further treatment. Care was taken to use alcohol samples from the same bulk for each comparative measurement. *cyclo*Hexane

was purified by treatment with oleum followed by percolation through silica gel, and the *n*-hexane was prepared from a suitable fraction of Fischer–Tropsch product.

*Preparation of Solutions.*—The purely alcoholic solutions and those containing lithium chloride were made from a common stock solution of the aromatic hydrocarbon in alcohol and in this manner errors in the preparation of the solution were minimized.

The actual concentrations of the hydrocarbons used are summarized in Table 2.

TABLE 2

| Hydrocarbon | | G./l. | Hydrocarbon | | G./l. |
|---|---|---|---|---|---|
| Benzene | | 0·350 | Anthracene | 240—260 mμ | 0·0005 |
| *m*-Xylene | | 0·170 | | 300—390 mμ | 0·010 |
| Naphthalene | 240—290 mμ | 0·025 | Chrysene | 250—280 mμ | 0·001 |
| | 290—320 mμ | 0·500 | | 280—330 mμ | 0·010 |
| | | | | 330—370 mμ | 0·050 |

*Spectrographic Measurements.*—All measurements were carried out on a Unicam Quartz Spectrophotometer, a hydrogen lamp being used a light source. The wave-length scale was checked by using a mercury lamp, and the wave-lengths recorded are corrected for the small deviations found. No error in the percentage absorption scale was detected at a wave-length of 546·1 mμ by using a set of neutral filters kindly lent by the Light Division, National Physical Laboratory.

*Four-cell Technique for measuring* $\Delta\varepsilon$.—The values of $\Delta\varepsilon$ were obtained by a direct comparison of the spectra of the aromatic hydrocarbon in one solvent with that in another. The four quartz cells employed were of 1-cm. path length and were used in matched pairs A,B; C,D. The procedure used to measure $\Delta\varepsilon$ for a lithium chloride solution was as follows. Cell A was filled with the required alcoholic lithium chloride solution containing the aromatic hydrocarbon, and cell C was filled with alcohol. Cell B was filled with an alcoholic solution of the aromatic hydrocarbon containing the same concentration of this material as in cell A. Cell D was filled with an alcoholic lithium chloride solution containing the same number of g. of salt per l. as the solution in cell A. Cells A and C were used together in the light beam, as were also cells B and D Thus the light path contained the same components at the same concentration when cells B and D were used together as when cells A and C were employed. The cell contents were interchanged after one measurement in order to minimize any small residual differences in the transmission of cell A plus cell C as compared with cell B plus cell D, and in order to minimize any differences in the path lengths of cell A and cell C, and of cell B and cell D. Thus the optical density scale of the spectrometer was set equal to 0·050 with cell B plus cell D as standard, and the optical density, $y_1$, of cell A plus cell C was measured. The solutions in cell A and cell B were interchanged, as also were the solutions in cell C and cell D, and a new difference reading, $y_2$, was found by taking the cell A plus cell C as standard. The value of the function $[(y_1 + y_2)/2 - 0{\cdot}050]$ was taken as the change produced by lithium chloride, and $\Delta\varepsilon$ calculated. All the difference values ($\Delta\varepsilon$) recorded in this paper are with respect to the alcoholic solutions as standards. The apparatus was not thermostatically controlled and the curves refer to a temperature of $20° \pm 3°$.

*Capillary-thickness Cell.*—The spectra of benzyl alcohol and of benzyl alcohol containing lithium chloride were obtained by means of a wedge cell. This was made from two thin quartz plates, gold leaf being used as a spacer at one end and with the other end of the wedge held together by a paper clip. Since the thickness of this cell could not be reproduced, the curves for benzyl alcohol and for 1N-lithium chloride in benzyl alcohol were plotted after arbitrarily adjusting the heights of the main peaks at 259 mμ to equality by multiplying all the values on one curve by a constant factor. No allowance was made for the small absorption produced by the salt itself at the small thickness employed.

CHEMICAL RESEARCH LABORATORY, TEDDINGTON. [*Received, March 21st*, 1952.]

## 601. *The Effect of Solvent on the Ultra-violet Absorption Spectra of Aromatic Hydrocarbons with Special Reference to the Mechanism of Salting-out. Part II.* Aqueous Salt Solutions.*

By E. F. G. Herington and W. Kynaston.

The spectra of solutions of benzene and naphthalene in alcohol and in water are compared, and a relation is indicated between the shapes of the absorption curves and the forces to which the aromatic hydrocarbons are subjected in solution.

The effects of lithium, sodium, and cæsium chloride on the spectra and solubility of benzene and of sodium chloride on the spectra of naphthalene in aqueous solutions have been studied. The spectra of saturated benzene solutions in water, 1N-sodium, 1N-lithium, and 1 or 2N-cæsium chloride are identical within the limits of experimental error. The spectra of the saturated benzene solution in 2N-sodium chloride exhibit smaller troughs, and the saturated solution in 2N-lithium chloride exhibits bigger troughs than the solution in pure water. The dependence of the form of the spectra on salt concentration for sodium chloride and for lithium chloride in water indicates that these salts produce more localised modifications in the structure of this solvent than does lithium chloride in alcohol. This conclusion is in agreement with evidence from the measurement of the entropy of the ions. The differences in the behaviour of these two salts are in harmony with published information on the structure of these solutions deduced from *P–V* data.

The changes produced in the spectra of benzene by sodium chloride and lithium chloride dissolved in various alcohol–water mixtures afford evidence of the preferential solvation of the ions by water. The effect of lithium chloride on the solubility of benzene and naphthalene and of sodium chloride on the solubility of benzene in alcohol–water mixtures is recorded.

The solubility of many aromatic hydrocarbons in water is sufficiently high to enable the ultra-violet absorption spectra to be measured by means of a modern spectrometer with cells as short as 1 cm. For example, Fig. 1 gives the spectra of benzene determined in this way in water and alcoholic solutions. The saturated aqueous solution was used to obtain this curve because unsaturated solutions of known composition could not be manipulated without some loss of hydrocarbon. The peaks at approximately 254 mμ for benzene have been adjusted to equality in this figure by multiplying all the values for one curve by a constant factor, with the result that the ordinate of this graph carries a purely arbitrary scale. The spectrum of aqueous benzene solutions was studied by Henri (*J. Phys. Radium*, 1922, **3**, 181), who employed a photographic technique and recorded only the wave-length of the absorption maxima. The positions of the maxima do not bear any relation to the dielectric constant of the solvent. The changes produced in the positions of the maxima by different solvents are not very large, as was commented upon by Scheibe (*Ber.*, 1926, **59**, 2617).

The majority of published theoretical treatments of solvent effects on absorption spectra have sought to correlate the shifts in the wave-length of the maxima with such properties of the pure solvent as refractive index or dielectric constant (see, *e.g.*, Badger and Pearce, *Spectrochim. Acta*, 1951, **4**, 280 for a review; see also Bayliss, *J. Chem. Physics*, 1950, **18**, 292). Some experimental evidence that systems conforming to these treatments do in fact exist has been obtained from a study of the spectra of aromatic hydrocarbons in a limited series of solvents (*e.g.*, Coggeshall and Pozefsky, *ibid.*, 1951, **19**, 980) but more detailed investigations have revealed many exceptions (*e.g.*, Maddams and Schnurmann, *ibid.*, 1949, **17**, 108; 1950, **19**, 1430). Since such theoretical treatments are based solely on the properties of the pure solvents and all specific interactions between solvent and solute are neglected, it is not surprising that many exceptions are found in practice because the observed shifts are probably the result of the superposition of several effects (see Bayliss,

* Part I, preceding paper.

*loc. cit.*). Thus, according to these theories the effect on the spectra of the forces which cause benzene and water to be only partly miscible and cause benzene and alcohol to be miscible in all proportions would be neglected. Further, these theories are usually concerned only with the position of the maxima and not with the general shape of the spectra. However, in Fig. 1 the shape of each curve is the striking feature and not the position of the maxima. There appears to be a general parallelism between the shape of the curve and the forces to which benzene molecules are subjected in solution. For instance, in alcohol, *cyclo*hexane, and *n*-hexane (see also Figs. 1 and 2, Part I), where the fugacity of the benzene is small, the spectra exhibit larger peaks and troughs than in water, where the fugacity is large. Naphthalene in water and in alcohol exhibits a similar behaviour qualitatively, but quantitatively the spectra show a smaller change in shape than does benzene in passing from a good solvent such as alcohol to a poor solvent such as water.

The spectra of saturated solutions of benzene in water, and in 1N-lithium, -sodium, and -cæsium chloride solutions were found to be indistinguishable when the peaks at 254 mμ were adjusted to equality by multiplying all the density values by the appropriate scale

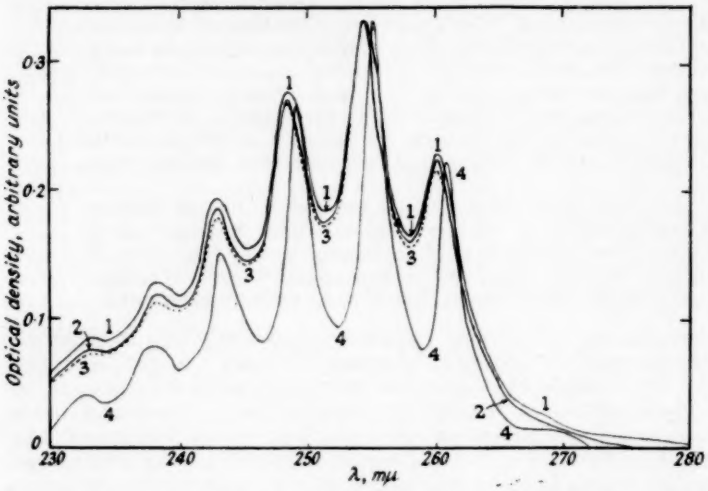



FIG. 1. *Benzene in various solvents.*

1, 2N-NaCl.
2, *Water.*
3, 2N-LiCl.
4, *Alcohol.*

factor. Fig. 1 shows the curves for benzene in 2N-sodium and -lithium chlorides. The spectrum of saturated solutions of naphthalene in 1N-sodium chloride was found to be indistinguishable from that of the hydrocarbon in pure water when the peaks were adjusted to equality at 276 mμ, while the curve for this material in 2N-sodium chloride lies slightly above that for this hydrocarbon in water. Clearly, the change in the shape of the curves for both of these hydrocarbons is not proportional to the salt concentration. A similar dependence of the wave-length of the maxima of acetone in aqueous solutions on the concentration of calcium, lithium, and hydrogen chlorides has been reported by Schiebe (*Ber.*, 1925, **58**, 586). The present results, taken in conjunction with the data for anthracene (250—260 mμ) given in Fig. 1, Part I, show that the dependence of the spectra on salt concentration is very different for the aqueous and the alcoholic solutions. These differences are rendered even more noteworthy when it is remembered that the aqueous solutions are saturated with respect to the hydrocarbon, so that the aromatic molecules will be forced into fairly close proximity to the ions, while the alcoholic solutions are unsaturated and the hydrocarbon molecules can occupy regions remote from the ions. This factor would be expected to make the spectral changes for the aqueous solutions relatively more sensitive to small salt concentrations than those for the alcoholic solutions—a conclusion which is, however, contrary to observation. Therefore it must be concluded that the ions produce less disturbance in the structure of water than in the structure of alcohol; indeed, in 1N-aqueous salt solutions there appear to be regions remote from the

ions where the solvent structure is little different from that in pure water. The conclusion that ions in water have a rather localised effect on solvent structure is in agreement with a tentative interpretation of the data on the hydration entropy of ions advanced by Eley and Pepper (*Trans. Faraday Soc.*, 1941, **37**, 581).

Cæsium chloride in 2N-aqueous solution is without measurable effect on the form of the spectra of benzene, and since the anions and cations in the alkali halide solutions probably act independently on the water structure, this observation indicates that the action of the chloride ion is much less than that of the lithium and sodium ions, a conclusion in agreement with the evidence obtained from transport-number measurements, etc. (*e.g.*, see Gross, *Chem. Reviews*, 1933, **13**, 91), on the behaviour of anions.

There is little information on the salting-out of aromatic hydrocarbons from aqueous solution. Gross (*loc. cit.*) has reported some unpublished work by Saylor which shows that 19·1% of the benzene is salted out by 0·5N-potassium chloride at 30° and some results by Saylor and Whitenfish which show that this hydrocarbon is salted out very little by cæsium chloride. The salting-out of benzene by sodium chloride at 20°±2° was found approximately to follow Setshenow's equation (*Ann. Chim. Phys.*, 1892, **25**, 226), *i.e.*, $\log(S_0/S) = kc$, where $S_0$ and $S$ are the solubilities of benzene in weight units per unit volume of water and salt solution respectively, $c$ is the salt concentration in moles/l., and $k$ is a constant. This constant for benzene is 0·18 unit, which may be compared with the following recorded values for other substances in sodium chloride solutions: benzoic acid 0·18 (Larsson, *Z. physikal. Chem.*, 1931, **153**, 306); helium 0·053, argon 0·058, hydrogen 0·095, oxygen 0·132, nitrous oxide 0·101, ethylene 0·093, ethyl acetate 0·166, phenol 0·220 (Harned and Owen, "The Physical Chemistry of Electrolytic Solutions," Table 12.10.5, Reinhold, 1950). The salting-out coefficient for benzene is therefore of approximately the same magnitude as for other substances of nearly the same molecular size (cf. benzoic acid and phenol).

The ratio $(S/S_0)$ for benzene has been determined for 1N-solutions of lithium, sodium, and cæsium chloride as equal to 0·75, 0·68, and 0·84, respectively, and for the first pair of these salts the values for naphthalene were 0·60 and 0·70. These salting-out ratios for benzene do not follow the order of the reciprocal of the ionic radii as required by Debye's theory. From Fig. 1 it can be seen that the spectra of the 2N-solutions of lithium and sodium chlorides differ markedly, and if the sizes of the troughs in the curves be interpreted in terms of the forces on the benzene molecule as suggested earlier, it follows that benzene in sodium chloride is subjected to greater forces, and in lithium chloride to smaller forces, than in water. "Salting-out," however, occurs for the lithium chloride solutions because, although the benzene molecules are subjected to smaller forces in the salt solution than in water, yet the aromatic molecules only occupy regions remote from the ions. The order of the salting-out effect for these halides and the behaviour of the spectra appear to be explicable in terms of the effect of the ions on the structure of water as discussed by Gibson (*Science Monthly*, 1938, **46**, 103) in connection with the compressibilities of salt solutions. He has concluded that the lithium ions are sufficiently small to fit into the orderly arrangement of the water molecules and actually make the openwork structure more rigid, while sodium ions, which are larger, break down the open water structure. The spectra in Fig. 1 agree qualitatively with this conception. The differences between the effects of N-lithium and N-sodium chloride on the solubilities of benzene (0·75 and 0·68) and of naphthalene (0·60 and 0·70) are presumably to be ascribed to a difference in the dependence of the solubilities of these materials on the structure of the solvent.

There is much experimental evidence from thermodynamic, refractometric, partial molar volume, and conductimetric studies that water tends to collect preferentially around the ions in alcohol–water mixtures (see, *e.g.*, Butler and Robertson, *Proc. Roy. Soc.*, 1929, *A*, **125**, 694; Shaw and Butler, *ibid.*, 1930, *A*, **129**, 519; Butler and Lees, *ibid.*, 1931, *A*, **131**, 382; Connell, Hamilton, and Butler, *ibid.*, 1934, *A*, **137**, 418). According to the electrostatic theory of salting-out, benzene should occupy regions of the solution remote from the ions in salt–alcohol–water mixtures, and hence the spectra of benzene should be modified by addition of salt if these regions are partly depleted of water by the salt. In order to see whether this effect could be detected spectroscopically, curve 3 in Fig. 2

was obtained, where the optical density at 257·8 mμ of various alcohol–water mixtures ($x$ is the mole-fraction of alcohol) containing 0·616 g. of benzene per l. is plotted against solution composition. In the same figure are plotted the corresponding quantities for benzene in 1N-sodium chloride solution for 0·00—0·30 mole-fraction of alcohol and for 1N-lithium chloride solution for the entire solvent range. The changes produced in the spectra of benzene by each of these salts for compositions 0—0·30 mole-fraction of alcohol are consistent with the supposition that benzene molecules in these salt solutions are surrounded by a solution richer in alcohol than the bulk composition. Sodium chloride produces a larger shift in the spectra than does lithium chloride for a given solvent composition, and this observation is probably to be ascribed to the greater affinity of lithium chloride for alcohol; *i.e.*, for a given initial solvent composition there are probably more alcohol molecules in the neighbourhood of a lithium than of a sodium ion.

The curve for the 1N-lithium chloride solution in Fig. 2 crosses that for the alcohol–water mixture alone at 0·50 mole-fraction of alcohol, which might be interpreted as indicating that the salt is preferentially solvated by alcohol in the alcohol-rich solutions; however, the optical density values for benzene in 1N-lithium chloride in 100% alcohol and for benzene in pure alcohol do not coincide (see values for $x = 1{\cdot}00$ in Fig. 2), showing that

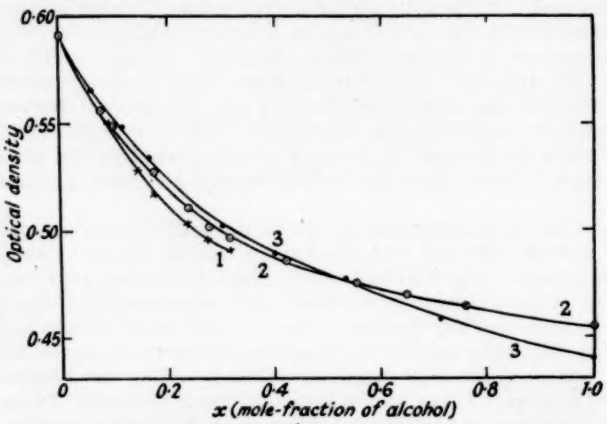



FIG. 2. *Benzene at* 257·8 mμ.
1, *Alcohol–*1N-NaCl.
2, *Alcohol–*1N-LiCl.
3, *Alcohol–water.*

this simple explanation is incorrect. The change in the spectra produced by the salt in 100% alcoholic solution is a result of a modification in the structure of the pure alcohol (see Part I), and a similar process probably complicates the interpretation of the spectral changes for alcohol–water mixtures of high alcohol content. This factor is probably not important for solutions of low alcohol content because, as Fig. 2 shows, all the curves coincide at $x = 0$. Hence, the curves in Fig. 2 for the composition range $x = 0$—0·30 can probably be interpreted solely in terms of the change in the water content of the "free" solvent, and the structure of the solvent in the neighbourhood of the aromatic molecules can be assumed to be the same as that of a solution of similar composition in the absence of salt.

Fig. 3 shows in more detail than Fig. 2 the effect of 1N-lithium chloride on the spectra of solutions containing 0·616 g. of benzene per l. dissolved in alcohol–water mixtures of 0·17, 0·27, and 1·00 mole-fraction of alcohol. Each $\Delta\varepsilon$ value was measured with respect to solvent of the same water–alcohol content. This figure strongly supports the conclusion reached in Part I that the effect of a salt on the spectrum of a dissolved aromatic hydrocarbon is due to a modification in the structure of the solvent, because if the effect had been due to direct electrical action of the ion on the hydrocarbon, all the curves in Fig. 3 would probably have been identical in form and the corresponding maxima and minima would have differed only in magnitude.

The preferential solvation of the ions by water should increase the concentration of alcohol in the free liquid of an alcohol–water mixture, and hence if a given quantity of

alcohol is a better solvent for a non-electrolyte in the enriched solution than is the original mixture, the addition of salt should cause " salting-in " of the non-electrolyte. In this manner Wright (*J.*, 1926, 1203) explained the observation that if 1-c.c. portions of benzene were added to 100 c.c. of 50% alcohol until the solution was turbid, then the addition of solid sodium chloride would clear the solution again. Recently, Bockris and Egan (*Trans. Faraday Soc.*, 1948, **44**, 151) have advanced a similar explanation to account for the effect

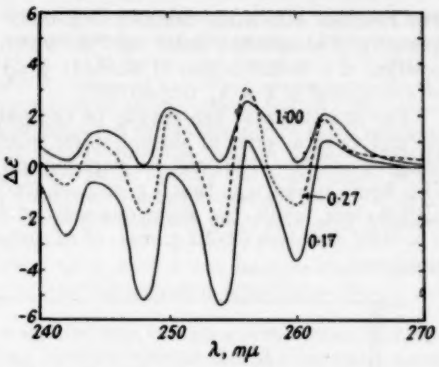



FIG. 3. *The effect of* 1N-*lithium chloride on the spectrum of benzene in various alcohol–water mixtures.*

(*The figures on each curve refer to mole-fraction of alcohol.*)

of sodium chloride on the solubility of benzoic acid in alcohol–water mixtures. The effect of 1N-lithium and 1N-sodium chloride on the solubility of benzene in alcohol–water mixtures in the range of solvent composition from 0·00 to 0·30 mole-fraction of alcohol, and also of 1N-lithium chloride on the solubility of naphthalene throughout the entire solvent range, has now been measured by a spectroscopic technique. The results of these measurements are summarised in Fig. 4, where the ratio $(S/S_0)$ is plotted against $x$, where $S$ is the solubility of the aromatic hydrocarbon per unit volume of the electrolyte solution, $S_0$ the solubility in the absence of salt, and $x$ the mole-fraction of alcohol. Thus in this figure " salting-out "

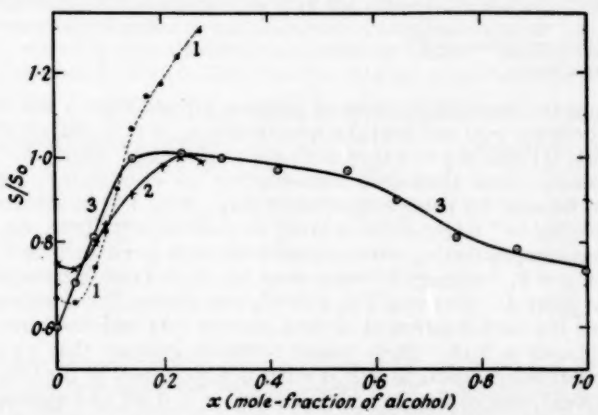



FIG. 4. *Relative solubilities of hydrocarbons in salt solutions.*

1, *Benzene in* 1N-NaCl.
2, *Benzene in* 1N-LiCl.
3, *Naphthalene in* 1N-LiCl.

corresponds to $(S/S_0) < 1$, and " salting-in " is equivalent to $(S/S_0) > 1$. These curves show that sodium chloride " salts-in " benzene much more readily than does lithium chloride, and indeed the ratio $(S/S_0)$ for lithium chloride only just exceeds 1 at $x = 0{\cdot}24$.

The discussion of the curves in Fig. 4 in terms of the solubilities of the aromatic hydrocarbon in alcohol–water mixtures in the absence of salt can most readily be carried out if a simple model of the system is adopted. Suppose a salt is dissolved in a quantity of solution containing 1 g.-mol. of alcohol of composition $x_1$ mole-fraction of alcohol, and let both water and alcohol be bound to the salt by solvation. Let a fraction $q$ of the alcohol present be

bound in this way, and let the resulting " free liquid " contain a mole-fraction $x_2$ of alcohol. Then if it be assumed that the water and alcohol bound to the ions are incapable of dissolving non-electrolyte while the " free liquid " has the same power of dissolving the non-electrolyte as an alcohol–water mixture of the same composition in the absence of salt, the following expression is obtained if volume changes on mixing are neglected:

$$(S/S_0) = (1 - q)\phi(x_2)/\phi(x_1) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

The function $\phi(x)$ is the number of g.-mols. of the non-electrolyte which dissolve in that quantity of an alcohol–water mixture which contains 1 g.-mol. of alcohol and has a composition of $x$ mole-fraction of alcohol; $\phi(x_2)$ and $\phi(x_1)$ are the values of the function $\phi(x)$ at $x = x_2$ and at $x = x_1$, respectively.

The function $\phi(x)$ can readily be computed from solubility measurements of the non-electrolyte in a series of alcohol–water mixtures, and the resulting functions for benzene (range $x = 0{\cdot}00$—$0{\cdot}30$) and for naphthalene (range $x = 0{\cdot}00$—$1{\cdot}00$) are plotted in Fig. 5. This figure shows, *e.g.*, that 1 g.-mol. of 100% alcohol ($x = 1{\cdot}00$) dissolves 0·035 g.-mol. of naphthalene, while the same quantity of alcohol mixed with 3 g.-mols. of water (*i.e.*, $x = 0{\cdot}25$) dissolves 0·0022 g.-mol. of naphthalene. The function $\phi(x)$ tends to infinity as $x$ tends to zero.

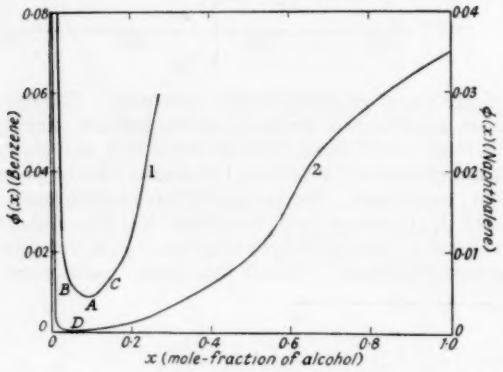



FIG. 5. *The function $\phi(x)$ plotted against $x$ for:*

1, *Benzene.*

2, *Naphthalene.*

The curves in Fig. 4 can now be discussed in terms of equation (1) and Figs. 2 and 5. Since Fig. 2 shows that for $x$ between 0·00 and 0·30 the relationship $x_2 > x_1$ is obeyed, it follows from Fig. 5 and equation (1) that if $q = 0$ then both salts will cause " salting-in " of benzene in solutions containing more than 0·10 mole-fraction of alcohol (*i.e.*, for points to the right of $A$, Fig. 5) because for these compositions $\phi(x_2)_1/\phi(x_1)$ will be greater than 1. Actually, if $q = 0$ " salting-in " might occur at lower alcohol concentrations, *e.g.*, at point $B$, Fig. 5, provided that composition $x_2$ corresponded with some point such as $C$, but on the present model when $q = 0$, " salting-in " *must occur* for alcohol concentrations greater than the composition at point $A$. However, Fig. 4 shows that lithium chloride does not produce " salting-in " until the mole-fraction of alcohol exceeds 0·22 and the corresponding value for sodium chloride is 0·13. These values therefore indicate that $q \neq 0$ for both of these salts and therefore some alcohol as well as water is immobilised by the ions. The difference between the solvent composition at which lithium ($x = 0{\cdot}22$) and sodium chloride ($x = 0{\cdot}13$) cause " salting-in " of benzene is to be ascribed to a higher value of $q$ for lithium than for sodium chloride. This difference in the behaviour of the two salts is presumably a result of the same forces which cause lithium chloride to be appreciably soluble and sodium chloride to be slightly soluble in 100% alcohol. The naphthalene curves in Figs. 4 and 5 also lead to the conclusion that for lithium chloride $q \neq 0$, because if $q = 0$ then " salting-in " would be expected for alcohol concentrations greater than 0·05 (Fig. 5), while, as Fig. 4 shows, this does not in fact take place until a mole-fraction of 0·12 is attained. The lower alcohol concentration at which " salting-in " by lithium chloride commences with naphthalene compared with benzene is compatible with the solubilities of these hydro-

carbons in the mixed solvents [*i.e.*, with the relative positions $D$ and $A$, of the minima in the $\phi(x)$–$x$ curves Fig. 5]. Therefore it may be concluded that lithium and sodium chlorides are preferentially solvated by water in alcohol–water mixture of 0 to 30% alcohol but the solvent sheaths contain some alcohol, and that of the lithium ion more than that of the sodium ion.

## Experimental

*Preparation of Compounds.*—The history of the majority of the compounds used has been given in Part I. " AnalaR " Sodium chloride was employed, and the cæsium chloride was a purchased specimen used without further purification. The water used was doubly distilled.

*Preparation of Solutions.*—Allowance was made by means of subsidiary experiments for the volume change accompanying mixing in the preparation of alcohol–water and alcohol–water–salt mixtures. Saturated solutions of benzene were prepared by shaking a very slight excess of liquid benzene with the solvent and keeping it in contact with the solution for several days. Saturated aqueous solutions of naphthalene were prepared by melting the hydrocarbon in the solvent, vigorously shaking it, and allowing the solutions to stand for several days. A small quantity of undissolved hydrocarbon was always present in the cell when saturated solutions were measured.

*Spectrographic Measurements.*—The equipment used in Part I was employed, and quartz cells of 1 and 0·3 mm. were used as required. The four-cell technique described in Part I was used to obtain the data for Figs. 2, 3, and 4.

*Salting-out Measurements.*—These values were obtained from the ratios of the optical densities of the saturated salt solution and of the saturated solution without salt at convenient wave-lengths: each optical-density measurement was corrected for the absorption of the corresponding solvent mixture. The difference produced in the shape of the spectra of the aromatic hydrocarbon by the salt was neglected for this purpose. The error in $(S/S_0)$ resulting from this approximation is probably less than 1% for the 1N-aqueous salt solutions and probably less than 5% for the 1N-alcoholic solutions. The actual temperatures used were not controlled and were $20° \pm 3°$, but the temperatures of any pair of saturated solutions of the same solvent composition, one with and one without salt, were the same.

*Solubility Measurements.*—These were obtained from the ratios of the optical densities, at a convenient wave-length, of the saturated solution to that of a synthetic mixture of approximately one-third of the concentration of the saturated solution. The values given in the following table were obtained in this manner for a temperature of $20° \pm 3°$.

The following solubilities are listed by Seidell (" Solubilities of Organic Compounds," Van Nostrand, New York, 1941, Vol. II): 100 g. of water dissolve 0·175 g. of benzene at 20°; 1 l. of

*Solubilities of benzene and naphthalene in alcohol–water mixtures.*

| Mole-fraction of EtOH in initial liquid | G./l. of satd. solution: $C_6H_6$ | G./l. of satd. solution: $C_{10}H_8$ | Mole-fraction of EtOH in initial liquid | G./l. of satd. solution: $C_6H_6$ | G./l. of satd. solution: $C_{10}H_8$ |
|---|---|---|---|---|---|
| 0 | 1·78 | 0·025 | 0·271 | 42·1 | — |
| 0·034 | 2·03 | 0·039 | 0·312 | — | 4·74 |
| 0·072 | 2·61 | 0·078 | 0·415 | — | 9·60 |
| 0·094 | 3·14 | — | 0·548 | — | 20·2 |
| 0·117 | 3·56 | — | 0·642 | — | 37·1 |
| 0·142 | 6·00 | 0·30 | 0·754 | — | 47·5 |
| 0·169 | 9·08 | — | 0·867 | — | 58·3 |
| 0·200 | 15·1 | — | 1·000 | — | 70·3 |
| 0·234 | 24·5 | 2·10 | | | |

water dissolves 0·022 g. of naphthalene at 15° and 0·030 g. at 20°; the solubility of naphthalene in alcohol is 10·2 g. per 100 g. of solvent at 20°. Andrew and Keefer (*J. Amer. Chem. Soc.*, 1949, **71**, 3644) have recorded that 1·74 g. of benzene and 0·0315 g. of naphthalene are dissolved in a litre of the saturated aqueous solutions at 20°. Recent measurements by Bohon and Claussen (*ibid.*, 1951, **73**, 1571) give a solubility of 1·79 g./l. for benzene and 0·028 g./l. for naphthalene at 20°. The figures in the above table are thus seen to be in satisfactory agreement with the published values.

The work described in this and the preceding paper has been carried out as part of the research programme of the Chemistry Research Board and is published by permission of the Director, Chemical Research Laboratory, Teddington.

Chemical Research Laboratory, Teddington. [*Received, March 21st*, 1952.]

## 602. *The Mercury Photo-sensitized Oxidation of Ethane and Methane.*

By J. A. Gray.

The experiments were designed to reproduce, and thus verify, the now conventional peroxide mechanism used to explain the low-temperature oxidation of hydrocarbons and to isolate the predicted product. The results are consistent with the mechanism proposed, *viz.*,

$$Hg(^1S_0) + h\nu = Hg(^3P_1) \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$Hg(^3P_1) + RH = Hg(^1S_0) + R + H \quad . \quad . \quad . \quad (2)$$

$$R + O_2 = R{\cdot}O{\cdot}O \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$R{\cdot}O{\cdot}O + RH = R{\cdot}O{\cdot}OH + R \quad . \quad . \quad . \quad . \quad (4)$$

where R represents $C_2H_5$ or $CH_3$. To prevent photochemical and thermal decomposition of the peroxide, a flow system was used at high rates of flow and low temperature (25°). The product condensable at −80° from the oxidation of ethane was ethyl hydroperoxide in a high state of purity. Methane gave a product which was predominately methyl hydroperoxide, formaldehyde being absent, thus excluding the possibility of the reactions

$$CH_3 + O_2 = H{\cdot}CHO + OH \quad (5)$$

and similar variants under the conditions of the present work and confirming that the suggested peroxide mechanism is operative.

The formation of hydroperoxides as intermediates has been postulated with increasing frequency during the last few years in order to obtain a reaction mechanism for the oxidation of organic compounds. Mulcahy (*Discuss. Faraday Soc.*, 1951, **10**, 259) has given a recent summary of the evidence for the formation of peroxides and extended the simplified theory of low-temperature oxidation as developed by Bardwell and Hinshelwood (*Proc. Roy. Soc.*, 1951, *A*, **207**, 461, 470) for hydrocarbons in the gas phase. As yet, however, experiments on gas-phase oxidations have not yielded peroxides in sufficient quantity or adequate purity to enable them to be isolated or identified, and there is still doubt as to the particular peroxide which is formed in a given set of circumstances in hydrocarbon combustion (Egerton, Emte, and Minkoff, *Discuss. Faraday Soc.*, 1951, **10**, 278). The object of the present investigation was to choose the oxidative conditions wherein a peroxide formed as an intermediate could survive and be recovered in a pure state for examination and identification. Photosensitized oxidation appeared to be the only satisfactory method, and ethane and methane were examined because of their position as the lowest members of the hydrocarbon series and because of the fundamental differences that have been suggested to explain their behaviour on oxidation: $C_2H_5 + O_2 = C_2H_5{\cdot}O{\cdot}O$ is not a difficult process to accept, but opinion has been more widely divided over the reaction $CH_3 + O_2$. Products suggested have been $H{\cdot}CHO + OH$ directly or through an intermediate energy-rich $CH_3{\cdot}O{\cdot}O$ molecule (Bates and Spence, *J. Amer. Chem. Soc.*, 1931, **53**, 1689; Norrish, *Rev. Inst. franç. Pétrole Ann. Combust. Liq.*, 1949, **4**, 288; Chamberlain and Walsh, *ibid.*, p. 301), $CH_3{\cdot}O_2$ (Raley, Porter, Rust, and Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 15), $HCO + H_2O$ (Marcotte and Noyes, *Discuss. Faraday Soc.*, 1951, **10**, 236), and it was hoped that the present work would enable this point to be settled for the particular conditions which had been chosen.

### Experimental

Commercial ethane, methane, and oxygen were used without further purification. The apparatus was similar to that used by Volman (*J. Chem. Physics*, 1946, **14**, 709), the arrangement being shown in Fig. 1. A metered hydrocarbon–oxygen mixture was dried by passage over Anhydrone and then passed over a number of mercury surfaces which were kept at 100° by water boiling under reflux. Two traps immersed in the water-thermostat acted as desaturators before the gas passed into the quartz spiral which surrounded the arc. The low-pressure mercury arc (type T/M5/369) was supplied by Messrs. Thermal Syndicate Ltd., and operated at 110 ma. from 1000 v A.C. Emission is unreversed at 2537 Å and, by running the

arc immersed in the thermostat and cooling the electrodes with a flow of compressed air, pressure broadening was reduced to a minimum and 1849-Å radiation was unlikely to reach the reaction spiral. The latter consisted of 6 turns of 9-mm. quartz tubing, the total volume being aprox. 92 c.c. The trap which followed the spiral was cooled on the outside by solid carbon dioxide–ethanol, and the centre tube was warmed by a jet of air. The annular space of approx. 1·5 mm. was found to be very efficient in condensing small quantities of product from a large volume of gas at rapid flow rates. At the end of an experiment this trap was isolated and residual hydrocarbon and oxygen were pumped off. The product was then subjected to fractionation under vacuum and was finally collected in a small tube which could be detached from the apparatus for weighing and for examination of the product.

*Results.*—1. *Ethane.* All experiments were carried out at a total gas pressure of 1 atm. This relatively high pressure eliminated unwanted side-reactions and prevented the quenching of excited mercury by the reaction products.

Trial experiments with a 50 : 50 $C_2H_6$–$O_2$ mixture suggested that at 25° conversion was very low, that the condensable product was mainly peroxide, and that ozone was also present, the formation of which was presumably initiated by the reaction

$$Hg(^3P_1) + O_2 = HgO + O \quad . \quad . \quad (6)$$

In reducing the effect of ozone to a minimum it was found that a 90 : 10 $C_2H_6$–$O_2$ mixture gave the optimum results, *i.e.*, a relatively pure product with no diminution of yield and with little or no ozone formation. Mercuric oxide was still found in the annular trap, but if ozone were formed it had no effect on the nature of the product, which appeared to be ethyl hydroperoxide. Further reduction of the oxygen to 5% hardly affected the yield or nature of the product. High flow rates had to be used to avoid photochemical decomposition of the peroxide, the total flow being 10—15 c.c./sec. On reducing this rate, aldehydes were detected in the carbon dioxide trap. An increase in flow rate did not affect the nature or amount of product, but of course the conversion was lower.

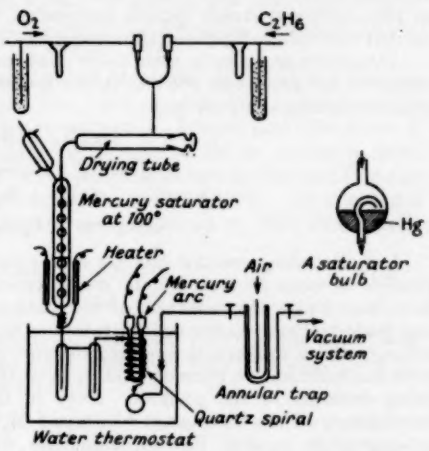



FIG. 1. *Experimental arrangement.*

Because of the unstable nature of the lower alkyl peroxides and the difficulty of finding suitable reagents to differentiate between them, the product was examined and identified by a variety of methods, namely, by titration, carbon and hydrogen analysis, micro-b. p., refractive index, vapour pressure, infra-red absorption, and polarography. On fractionation and melting it appeared to consist of a single compound (Found : C, 39·2; H, 9·7. Calc. for $C_2H_6O_2$ : C, 38·7; H, 9·7%). The micro-b. p. was approx. 90—91°, extrapolation of the vapour-pressure measurements of Egerton, Emte, and Minkoff (*loc. cit.*) giving 95°; $n_D^{20}$ was 1·388 as against their value of 1·380. Iodine liberation was 101—103% of theory; it was carried out by weighing about 0·1 g. of product, making it up to a known volume, adding glacial acetic acid and sodium hydrogen carbonate to a portion, and keeping it in the dark in a loosely stoppered bottle for 10 minutes. Saturated potassium iodide solution was added, and after standing in the dark for a further 15 minutes, the solution was diluted and titrated with 0·1N-sodium thiosulphate, with starch as indicator. Trial experiments showed that this method, which is basically the same as that of Harris (*Proc. Roy. Soc.*, 1939, *A*, **173**, 126), gave reproducible results.

Polarographic examination was best carried out in 0·1N-lithium chloride solution (Stern and Polak, *Acta Physicochim.*, 1939, **11**, 797). A Cambridge polarograph was used, and hydrogen was bubbled through the solution under examination for 2 minutes to eliminate the oxygen wave. The reduction potential of the product was found to be 0·25—0·3 v, which is the same as that given by Stern and Polak for $C_2H_5{\cdot}O{\cdot}OH$ and is well removed from the value of 0·5 v quoted by the same authors for $C_2H_5{\cdot}O{\cdot}O{\cdot}C_2H_5$. Aldehydes were absent.

Vapour-pressure measurements on the product confirmed that of the two most probable compositions, *i.e.*, $C_2H_5{\cdot}O{\cdot}OH$ pure, or containing an equimolar mixture of $C_2H_5{\cdot}O{\cdot}OC_2H_5$ and $H_2O_2$, the former was the only possible one. The vapour pressure at −16° and 0° coincided

exactly with that reported by Egerton, Emte, and Minkoff (*loc. cit.*) for $C_2H_5{\cdot}O{\cdot}O{\cdot}H$, being 1·5 and 4·7 mm., respectively, in contrast with 28·4 and 63·1 mm. for $C_2H_5{\cdot}O{\cdot}O{\cdot}C_2H_5$.

The published data on the absorption spectra of the lower alkyl peroxides are scanty. Egerton, Harris, and Young (*Trans. Faraday Soc.*, 1948, **44**, 745) have re-examined the ultra-violet absorption spectra of peroxides but the method cannot be used for the positive identification of an unknown. The infra-red absorption of $C_2H_5{\cdot}O{\cdot}OH$ does not appear to be recorded in the literature but Dr. G. J. Minkoff has kindly made available his unpublished collection of infra-red absorption spectra of organic peroxides, including that of $C_2H_5{\cdot}O{\cdot}OH$ in solution. He has described the double-beam instrument which he used (*Fuel*, 1950, **39**, 228).

The infra-red absorption of the liquid product was examined on a Grubb-Parsons single-beam instrument with a rock-salt prism and A.C. amplification; the spectrum obtained was nearly identical with that obtained by Minkoff for $C_2H_5{\cdot}O{\cdot}OH$, due allowance being made for the different conditions obtaining. In contrast to the absorption of $C_2H_5{\cdot}O{\cdot}O{\cdot}C_2H_5$ were noticed: (*a*) the absence of an intense band at ~880 cm.$^{-1}$ found by Minkoff and tentatively attributed by Leadbeater (*Compt. rend.*, 1950, **230**, 829) to the RO—OR linkage, and (*b*) the presence of a band at 783 cm.$^{-1}$ and strong general absorption below about 900 cm.$^{-1}$ which were not given by diethyl peroxide. No detectable amount of ethanol was present.

Thus there seems to be little doubt that the main product of the photosensitized oxidation of ethane at 25° and under the conditions described is ethyl hydroperoxide, in agreement with the reaction mechanism proposed:

$$Hg(^1S_0) + h\nu = Hg(^3P_1) \quad . . . . . (1)$$

$$Hg(^3P_1) + C_2H_6 = Hg(^1S_0) + C_2H_5 + H \quad . . . . . (7)$$

$$C_2H_5 + O_2 = C_2H_5{\cdot}O{\cdot}O \quad . . . . . (8)$$

$$C_2H_5{\cdot}O{\cdot}O + C_2H_6 = C_2H_5{\cdot}O{\cdot}OH + C_2H_5 \quad . . . . . (9)$$

A chain-ending reaction has not been proposed in this qualitative study as it has not been possible to make accurate kinetic measurements. The limit set on the conversion by the high flow rates makes the detection of non-condensable products extremely difficult. An attempt was made to build up the concentration of these products by using a circulating system which incorporated a diaphragm-operated mercury-piston type pump, but the flow rates obtainable were not sufficient to prevent photolysis of the peroxide, formaldehyde and a higher aldehyde being detected in the product. Even in the case of the normal flow system, when the temperature of the thermostat was raised to 50° aldehyde was detected in the product by the polarographic method but formaldehyde was absent. This indicated that heterogeneous thermal decomposition of the peroxide was commencing. An approximate calculation of the quantum yield of $C_2H_5{\cdot}O{\cdot}OH$ formation based on the known 2537-Å emission of the arc indicates that very short chains are involved.

The rate of production of $C_2H_5{\cdot}O{\cdot}OH$ is shown in the table, where, owing to variations in pumping, etc., the weight of product refers to the maximum obtained and not to the mean of several runs.

*The variation of yield with reaction conditions.*

| Run | Conditions | | $C_2H_5{\cdot}O{\cdot}OH$ produced, g./h. |
|---|---|---|---|
| 6A/94 | 90 : 10 $C_2H_6$–$O_2$ | 25° | 0·012 | |
| 6A/93 | ,, | 40 | 0·036 | Product contained approx. |
| 6A/101 | ,, | 50 | 0·039 | 7% of $CH_3{\cdot}CHO$ |
| 6A/107 | 95 : 5 $C_2H_6$–$O_2$ | 25 | 0·014 | |

2. *Methane.* The product of the photosensitized oxidation of methane, carried out under the conditions already described for ethane, was examined by similar methods and appeared to be mainly $CH_3{\cdot}O{\cdot}OH$. Properties were as follows: $n_D^{20}$ 1·374; micro-b. p. 88—89° (Egerton, Emte, and Minkoff give 1·364 and 86°) (Found: C, 29·1; H, 9·6. Calc. for $CH_4O_2$: C, 25·0; H, 8·3%). Its polarographic behaviour was the same as that reported by Stern and Polak (*loc. cit.*) for $CH_3{\cdot}O{\cdot}OH$, and aldehydes were absent. It was undoubtedly peroxidic in nature, but iodine liberation was only 60% of theory, and although a low result is often to be expected, it is probable that an impurity was present. This might be $CH_3{\cdot}O{\cdot}OCH_3$, $CH_3{\cdot}OH$, and/or $H_2O_2$. The infra-red absorption spectrum was in fair agreement with that of the $CH_3{\cdot}O{\cdot}OH$ spectrum provided by Minkoff, but the possibility of the presence of up to 30% of $CH_3{\cdot}OH$ could not be excluded. The absorption spectrum of $CH_3{\cdot}O{\cdot}O{\cdot}CH_3$ was not available and $CH_3{\cdot}O{\cdot}OH$ could not be differentiated from $H_2O_2$ on the polarograph.

Although the composition of the whole of the product is not known with certainty, the significant conclusion to be drawn is that it contained at least 50% of methyl hydroperoxide but no formaldehyde. Trace amounts of formaldehyde were detected in the carbon dioxide trap when it was washed out with water after a run, and may have been produced from the decomposition of peroxide in the presence of mercury even at −80° or during vacuum distillation. The maximum yield of product was 0·0129 g./h. at 40° and 0·0106 g./h. at 25°.

## Discussion

*Ethane.*—It appears that the peroxide mechanism applied to the photosensitized oxidation of ethane gives a prediction which has been fulfilled by experiment. A variant which might have been applied to the mechanism, *viz.*, $C_2H_5{\cdot}O{\cdot}O + C_2H_6 = C_2H_5{\cdot}O{\cdot}O{\cdot}C_2H_5 + H$, does not take place to any significant extent under the conditions of the present work. The absence of detectable amounts of water or hydrogen peroxide in the product indicates that at the temperatures used hydrogen atoms are not removed by way of $H + O_2 = HO_2^* \longrightarrow H_2O$ or $H_2O_2$, and that radical recombination or abstraction of hydrogen from ethane is of greater importance. Both radicals being comparatively stable, a possible chain-ending step is the reaction $C_2H_5{\cdot}O{\cdot}O + HO_2 = C_2H_5{\cdot}O{\cdot}OH + O_2$.

The course of the reaction is in general similar to that obtaining in the photochemical oxidation of olefins (Bateman and Gee, *Proc. Roy. Soc.*, 1949, *A*, **195**, 376). Reaction (7) is that expected from the findings of Steacie and his co-workers (" Atomic and Free Radical Reactions," Reinhold Publ. Corpn., N.Y., 1946), although it is at variance with the conclusions of Nalbandyan, who has investigated the photosensitized oxidation of ethane (*Compt. rend. U.R.S.S.*, 1949, **66**, 413) and of methane (*ibid.*, 1948, **60**, 607). He concludes from the $H{\cdot}CHO/CH_3{\cdot}CHO$ ratio in his product, with a flow-system at 300°, that of the two reactions

$$C_2H_6 + Hg(^3P_1) = CH_3 + CH_3 + Hg(^1S_0) \quad . \quad . \quad . \quad . \quad . \quad (10)$$

$$C_2H_6 + Hg(^3P_1) = C_2H_5 + H + Hg(^1S_0) \quad . \quad . \quad . \quad . \quad . \quad (7)$$

the former is the faster. This was not the finding of Bywater and Steacie (*J. Chem. Physics*, 1951, **19**, 326) who found the reverse to hold, and may easily be explained by the homogeneous decomposition of the hydroperoxide :

$$C_2H_5{\cdot}O{\cdot}OH = C_2H_5{\cdot}O + OH \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

$$C_2H_5{\cdot}O = CH_3 + H{\cdot}CHO \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

$$C_2H_5{\cdot}O = H + CH_3{\cdot}CHO \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

It has been found (Gray, Thesis, London, 1950) that ethoxy-radicals generated by the photolysis of ethyl nitrate can decompose by the two alternative methods; the bond dissociation energies were calculated to be 11 kcal. for (12) and 13 kcal. for (13). Estimated activation energies were 21 and 23 kcal., respectively.

*Methane.*—Of greatest interest in the photosensitized oxidation of methane at temperatures up to 50° is that the two variants on the peroxide mechanism, *viz.*,

$$CH_3 + O_2 = H{\cdot}CHO + OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

and

$$CH_3 + O_2 = H{\cdot}CO + H_2O \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

are quite definitely excluded. This result is not unexpected, for neither (5) nor (14) involves simple reaction steps, both requiring drastic rearrangement of inter-atomic linkages. The initial step (Steacie, *op. cit.*)

$$CH_4 + Hg(^3P_1) = CH_3 + H + Hg(^1S_0) \quad . \quad . \quad . \quad . \quad . \quad (15)$$

being assumed, the impurities in the product indicate that there is a possibility that

$$CH_3 + O_2 = CH_3O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (16)$$

is followed by

$$CH_3{\cdot}O{\cdot}O + CH_4 = CH_3{\cdot}O{\cdot}O{\cdot}CH_3 + H \quad . \quad . \quad . \quad . \quad . \quad (17)$$

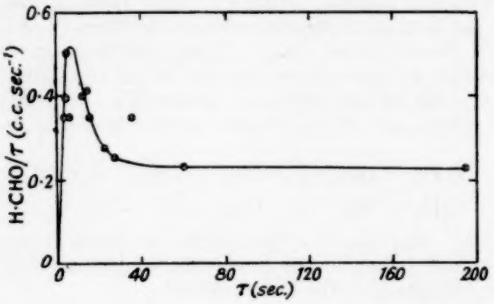
in addition to

$$CH_3{\cdot}O{\cdot}O + CH_4 = CH_3{\cdot}O{\cdot}OH + CH_3 \quad . \quad . \quad . \quad . \quad . \quad (18)$$

The product may undergo further heterogeneous decomposition even in the carbon dioxide trap, particularly in the inevitable presence of mercury before the separation by vacuum fractionation.

Nalbandyan has used equation (5) to interpret his results on the photosensitized oxidation of methane. However, it is considered that the peroxide mechanism may equally well be applied to explain his results. If $CH_3{\cdot}O{\cdot}OH$ is an intermediate, then the quantum yield of formaldehyde should be zero at infinitely rapid flow rate, should rise rapidly to a maximum with decomposition of $CH_3{\cdot}O{\cdot}OH$, and then fall off quasi-exponentially at longer contact time, to a value dependent on the proportion of oxygen in the reactants, owing to thermal and photochemical oxidation of formaldehyde itself. In Fig. 2, $H{\cdot}CHO/\tau$ is plotted against $\tau$ from values given by Nalbandyan (*loc. cit.*, 1948) for $H{\cdot}CHO$ (total $H{\cdot}CHO$ produced, in c.c.) and $\tau$ (time of residence in the illuminated zone, in sec.) in runs carried out at 400° on equal volumes of reacting material. It will be seen that a curve of the shape described above for $CH_3{\cdot}O{\cdot}OH$ as an intermediate fits the experimental data with only one major deviation, whereas a curve representing monotonic decay of formaldehyde from a positive intercept on the $H{\cdot}CHO/\tau$ axis would involve a considerable amount of scatter.

FIG. 2.




Thus it is possible that even at 400° the thermal oxidation of methane proceeds in a manner which can be explained on the assumption of an intermediate hydroperoxide with subsequent oxidation of its decomposition products :

$$CH_3 + O_2 = CH_3{\cdot}O{\cdot}O \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (16)$$

$$CH_3{\cdot}O{\cdot}O + CH_4 = CH_3{\cdot}O{\cdot}OH + CH_3 \quad . \quad . \quad . \quad . \quad . \quad . \quad (18)$$

$$CH_3{\cdot}O{\cdot}OH = CH_3{\cdot}O + OH \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (19)$$

$$CH_3{\cdot}O = H{\cdot}CHO + H \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (20)$$

$$H{\cdot}CHO + O_2 \longrightarrow \text{Products}$$

$$CH_3{\cdot}O{\cdot}OH + O_2 \longrightarrow \text{Products}$$

Reaction (16) is also supported by the work of Raley, Porter, Rust, and Vaughan (*loc. cit.*) who caused oxygen to react with methyl radicals produced from the thermal decomposition of di-*tert.*-butyl peroxide. In their case the $CH_3O_2$ radicals were in high concentration and did not abstract a hydrogen atom from di-*tert.*-butyl peroxide and disproportionation occurred : $CH_3O_2 + CH_3O_2 = 2CH_3O + O_2$. It may be that a similar process in the present work leads to the formation of methanol by the reaction $CH_3O + CH_4 = CH_3{\cdot}OH + CH_3$, as investigations on the photolysis of methyl nitrite (Gray, *op. cit.*) indicate that the $CH_3O$ radical may be comparatively stable up to temperatures of well above 100°.

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## 603. *Inorganic Chromatography on Cellulose. Part XII.* The Quantitative Determination of Tantalum and Niobium in Complex Minerals, Ores, and Synthetic Materials.*

By A. F. Williams.

A new chromatographic method based on cellulose as adsorbent and ethyl methyl ketone containing hydrofluoric acid as solvent, has been described (Part XI *) for the quantitative separation of tantalum from niobium and for their determination in certain types of mixtures. This method has been modified so that it is now applicable to the quantitative determination of these elements in complex materials containing appreciable amounts of titanium, tin, and zirconium. A solution of the sample in hydrofluoric acid containing ammonium fluoride being used, tantalum is extracted first in ethyl methyl ketone saturated with water. The column is then washed with ketone containing 1% (v/v) of hydrofluoric acid (40% w/w aqueous solution) which arrests the movement of titanium, tin, and zirconium. Niobium is then extracted with the ketone containing 12·5% of hydrofluoric acid. After removal of solvent the tantalum and niobium are determined in the appropriate fractions by ignition to the pentoxides.

The procedure is simple and accurate and has been used for the analysis of a wide range of natural and synthetic materials.

In Part XI * a method was described for the quantitative separation of tantalum from niobium by chromatographic extraction of the fluorides in the presence of ammonium fluoride, cellulose adsorbent and ethyl methyl ketone solvents being used. Tantalum was extracted first with ethyl methyl ketone saturated with water; niobium was then extracted with ethyl methyl ketone containing 7·5% (v/v) of hydrofluoric acid (40% aqueous solution). The same procedure was applied to minerals and ores but, whereas the tantalum fraction was obtained free from other ions, the niobium fraction was contaminated with appreciable amounts of titanium, tin, and zirconium when these metals were present in the original sample. The degree of contamination was reduced by re-extraction of the niobium fraction, but it was realised that a simple modification of the procedure was desirable if it was to be of general application in earth-acid analysis, for tantalum and niobium frequently occur in association with titanium, tin, and zirconium.

A modification of the above procedure has now been found so that niobium can be extracted free from titanium, tin, and zirconium.

It has been shown that extraction with ethyl methyl ketone containing 2% of hydrofluoric acid after extraction of tantalum prevents movement of titanium, etc., in the solvent subsequently employed for niobium (ethyl methyl ketone containing 7·5% of hydrofluoric acid) when this element is absent. When niobium was present, however, these elements were partially extracted but to a less degree than when the intermediate solvent was omitted; also a much larger volume of solvent was required to effect complete extraction of niobium (*J.*, 1952, 1504). The reduction in movement of titanium, etc., under these modified conditions appeared to be due to removal of water from the cellulose by the low-acidity solvent, the presence of water (in addition to hydrofluoric acid) in the solvent being necessary for extraction of these elements. Further research on these lines has led to modifications such that the procedure can now be applied to a wider range of materials than was hitherto possible. The new chromatographic procedure involves the following operations: (*a*) Tantalum is extracted with the solvent originally employed (*J.*, 1952, 1497) but the volume has been reduced to 250 ml., no advantage being gained by employing a greater amount. (*b*) The column is next conditioned by washing it with 400 ml. of ethyl methyl ketone containing 1% of hydrofluoric acid (40% aqueous solution). In this way, the quantity of water present in the cellulose is reduced to a value which is too small to permit extraction of titanium, tin, and zirconium by the solvent next employed for

* Part XI, *J.*, 1952, 1497.

extraction of niobium. (*c*) Niobium is extracted with a solvent consisting of ethyl methyl ketone containing 12·5% of hydrofluoric acid instead of 7·5% as originally used and amounts equivalent to 200 mg. of niobium metal are readily extracted in a volume of *ca.* 400—500 ml. (*d*) The earth acids are finally determined as oxides in the first and the last fraction respectively, by ignition of the residual solutions obtained after removal of the solvent. The entire process is simple and can be carried out by a relatively unskilled analyst.

Although the types of complexes formed in the chromatographic system have not yet been intensively studied, the following conditions seem to be involved. (*a*) Tantalum is extracted as fluoride by ethyl methyl ketone saturated with water but free from added hydrofluoric acid. Niobium is not extracted, neither are commonly occurring metal ions such as Th, Ce, lanthanons, Fe, Al, Mn, Cu, Ni, Ti, Sn, Zr, W, Pb, Ca, Mg, etc. (*b*) Increase in concentration of hydrofluoric acid and water in the solvent favours extraction of Nb, Ti, Sn, Zr, and small amounts of metal ions which form soluble fluorides. (*c*) A low concentration (*e.g.*, 1%) of hydrofluoric acid in the solvent and a very low concentration of water give unfavourable conditions for extraction of niobium and other metal ions; hence the use of such solvent for removal of water after extraction of tantalum. (*d*) Increase in concentration of hydrofluoric acid in the solvent favours extraction of niobium. Provided that water is absent or present in only a very low concentration, as when the intermediate solvent (1% of hydrofluoric acid) has previously been used, metal ions other than niobium are not extracted (except tungsten, which is partly extracted).

The procedure has now been extensively applied to complex synthetic materials (*e.g.*, ferro–tantalum–niobium alloys) and minerals such as pyrochlore and columbite. It has been shown that in many instances, particularly in the presence of certain foreign ions, there is a small hold-up of tantalum during its extraction by the water-saturated solvent. This was recovered by re-extraction of the niobium fraction (*J.*, 1952, 1504). Improvements in technique have reduced this effect, and it has been completely overcome by making a simple acid hydrolysis on the sample, before chromatographic extraction. This was found to be necessary only when very high accuracy was required, since the extent of the hold-up was usually less than 2% of the amount present in the original sample. This preliminary chemical step was also employed when the earth acids present in the sample were less than about 1%. The analysis of low-grade ores and the treatment of phosphatic materials will form the subject of a paper shortly to be submitted.

So far, the only metal ion which has caused difficulty is tungsten, because it is partly extracted with the niobium. This metal is easily determined in the ignited niobium pentoxide by the colorimetric thiocyanate procedure. In the early work on the separation of tantalum from niobium it was found that when tantalum was present in excess of niobium it was partly retained in the cellulose wad (*J.*, 1952, 1497). This peculiarity is being reinvestigated and the first experiments showed that tantalum was quantitatively recovered from a mixture containing 150 mg. of Ta and 50 mg. of Nb. Such results are no doubt due to improvements in the chromatographic technique since the method was first devised. Further investigations on high-grade tantalum ores are continuing.

## Experimental

(1) *Study of the Movements of Titanium, Tin, and Zirconium under the Solvent Conditions employed for Extraction of Niobium.*—Early experiments had shown that the best conditions for the preparation of the sample solution from the material under analysis before transfer to the chromatographic column, involved a final solution in 8 ml. of dilute hydrofluoric acid (25% of 40% w/w solution) and addition of 1 g. of ammonium fluoride. The addition of the latter was found to be essential in order to obtain complete extraction of the earth acid fluorides and it was also essential as complexing agent for certain impurities such as titanium and tin. Time has not permitted an intensive study of its exact rôle in the chromatographic process. The solvent originally employed for extraction of tantalum (ethyl methyl ketone saturated with water) has not been changed, since almost complete extraction of tantalum with a high degree of purity can be obtained from extremely complex mixtures. Variations in solvent conditions which have been carried out in order to find a suitable modification which would lead to extraction of niobium without extraction of impurities were always made after the extraction of

tantalum, and even in its absence the solvent used for tantalum extraction was always first passed through the column.

A number of experiments was carried out in order to observe whether additional ammonium fluoride in the sample solution would lead to complete retention of titanium, etc., and at the same time the effect on extraction of niobium was examined. The results of these experiments showed that increase in concentration of ammonium fluoride brought about a more rapid extraction of niobium in the earlier stages of the extraction with the solvent (ethyl methyl ketone containing 7·5% of hydrofluoric acid) but a volume of 400—500 ml. was still required to effect complete extraction. Under these conditions there was no effect on the extraction of impurities (titanium, etc.) but tantalum proved more difficult to extract with the water-saturated solvent. All further experiments were therefore made with sample solutions containing 1 g. of ammonium fluoride.

Further experiments were carried out upon a complex mixture (concentrate V) containing titanium, tin, and zirconium in addition to other metal ions, both with and without added niobium. The metals and ratios in which they were present are as follows: $TiO_2$, 30; $SnO_2$, 15; $ZrO_2$, 10; $MnO_2$, 5; $Fe_2O_3$, 5; $WO_3$, 10 parts.

0·07 G. of this mixture being used, about 40% was extracted in the first 150 ml. of the solvent first used for niobium extraction (ethyl methyl ketone containing 7·5% of hydrofluoric acid) with only slight extraction in later fractions. The ignited oxides from this extraction contained about 30% of titania with tin, zirconium, and tungsten forming the major constituents of the residual elements. A similar experiment was carried out with addition of about 200 mg. of niobium (as metal) to the same amount of concentrate V. Approximately half the niobium was extracted in the first 150 ml. of solvent. This niobium fraction contained about 30% of the original concentrate (mainly $TiO_2$, $SnO_2$, $ZrO_2$, and $WO_3$). Niobium extracted in later fractions was found to be very pure, and this gave an important clue to the mechanism of the movement of other metals and confirmed that water played an important part in the movement of impurities. Experiments were made in which the effect of hydrofluoric acid concentration in the solvent, and the volume of solvent employed, on the movement of impurities and extraction of niobium were studied. In this way, it was hoped to find the lowest concentration of hydrofluoric acid in the solvent and the volume required to arrest movement of impurities without causing extraction of niobium.

(2) *Extraction of Niobium and Retention of Impurities. Study of Volume and Acidity of Ethyl Methyl Ketone Solvent.*—In the following experiments, the sample solution was prepared in the manner described on p. 3161, where a full description of the method is given. The solvent used for tantalum extraction was always employed before using the solvent containing hydrofluoric acid. A known weight of concentrate V being used, usually about 70 mg. (see above for composition), extractions were carried out after the addition of niobium to the sample. Extractions were made in the manner described on p. 3161. Niobium and any metal ions, if extracted, were determined by direct ignition to oxide after removal of solvent (see p. 3162). The oxides so obtained were analysed colorimetrically for titanium (and sometimes tungsten), since this metal also gave an indication of the behaviour of tin and zirconium but was more easily determined. Spectrographic analyses were also made on a number of the fractions.

In the experiments which follow (Table 1) tantalum was extracted with 250 ml. of ethyl methyl ketone, saturated with water. Hydrofluoric acid was then added to the dry solvent, and in the different experiments the concentration was progressivly decreased from 3·5% v/v of acid (40% w/w aqueous solution) and a given volume of such solvent was passed through the column. After collection of the eluant in fractions, niobium was extracted with solvent of higher acidity. The results of spectrographic analyses are shown in Table 2.

Comparison of the results obtained shows that the progressive reduction of acidity of the intermediate solvent from 3·5% to 1% causes a decrease in the amount of niobium extracted and also in the amount of impurities. The results show that, in order to extract niobium in a reasonable volume of solvent (400—500 ml.), after use of the intermediate solvent, a higher concentration of hydrofluoric acid than that hitherto employed is required for complete extraction of niobium.

The procedure used in experiment (*d*) was adopted, apart from minor modifications, in all further experiments and applications. It is fully described on p. 3163. The only impurity in the extracted niobium, for which a correction needs to be applied, is tungsten, when present in the original sample.

(3) *Application of the New Chromatographic Procedure to the Determination of Tantalum and Niobium in Synthetic Mixtures.*—Having established the conditions for obtaining pure niobium

(except for tungsten) from a complex base mixture, we examined the same conditions for extraction of niobium in the absence of impurities. It was known that impurities could alter the behaviour of niobium fluorides on extraction with ethyl methyl ketone–hydrofluoric acid solvents.

TABLE 1.

| Expt. | Wt. of concentrate V, mg. | Wt. of Nb, mg. | Solvent COMeEt : HF, % | No. of fraction | Vol. of fraction, ml. | Wt. of oxides derived from fraction, mg. | | $TiO_2$ content of oxides (colorimetric determinations), mg. | |
|---|---|---|---|---|---|---|---|---|---|
| (*a*) | 70 | 205 | 3·5 | 1 | 100 | <1 | | — | |
| | | | ,, | 2 | 100 | 89·3 | | 2·7 | |
| | | | ,, | 3 | 100 | 39·2 | | 2·8 | |
| | | | ,, | 4 | 100 | 24·3 | | <0·02 | |
| | | | 7·5 | 5 | 200 | 116·9 | | 0·03 | |
| (*b*) | 70 | 190·4 | 2·5 | 1 | 200 | nil | | — | |
| | | | ,, | 2 | 100 | 20·0 | | 1·6 | |
| | | | ,, | 3 | 100 | 4·0 | | nil | |
| | | | ,, | 4 | 100 | 3·3 | | nil | |
| | | | 7·5 | 5 | 200 | 73·6 | | nil | |
| | | | ,, | 6 | 200 | 111·3 | | nil | |
| | | | ,, | 7 | 150 | 44·3 | | nil | |
| | | | ,, | 8 | 50 | 9·6 | | nil | |
| | | | | | | 266·1 | (≡ 186·3 mg. Nb) | | |
| (*c*) | 70 | 201·5 | 1 | 1 | 200 | trace | | — | |
| | | | ,, | 2 | 100 | ,, | | — | |
| | | | ,, | 3 | 100 | ,, | | — | |
| | | | 10 | 4 | 200 | 208·0 | | nil | |
| | | | ,, | 5 | 200 | 73·1 | 200·5 mg. Nb | nil | |
| | | | ,, | 6 | 200 | 5·2 | | nil | |
| | | | ,, | 7 | 100 | 2·0 | | — | |
| | | | | | | | | $TiO_2$, mg. | $WO_3$, mg. |
| (*d*) | 80 | 193·5 | 1 | 1 | 400 | nil | | — | — |
| | | | 12·5 | 2 | 200 | 192·7 | 193·2 mg. Nb | nil | — |
| | | | ,, | 3 | 100 | 69·7 | after correction for W | nil | 0·8 |
| | | | ,, | 4 | 100 | 14·4 | | nil | 0·6 |
| | | | ,, | 5 | 100 | 1·4 | | — | 1·1 |

*Note.*—The volume of low-acidity solvent was not reduced below 400 ml. in experiment (*d*) since preliminary experiments had shown that a smaller volume could lead to subsequent extraction of impurities in the solvent employed for niobium.

TABLE 2.

| | | Amount of impurity in separated niobium oxide : | | | | | |
|---|---|---|---|---|---|---|---|
| Expt. | Fraction | $Fe_2O_3$, % | MnO, % | $SnO_2$, % | $TiO_2$, % | $ZrO_2$, % | $WO_3$ % (or mg. in whole fraction) |
| (*a*) | 2 | 1·0 | 0·1—1·0 | 0·3—1·0 | >5·0 | 1·0—3·0 | — |
| | 5 | 0·03—0·1 | <0·1 | 0·01 | 0·03 | <0·1 | 1·1% |
| (*b*) | 5 | 0·03—0·1 | <0·1 | <0·01 | 0·1—0·03 | <0·3 | — |
| | 6 | 0·03—0·1 | <0·1 | <0·01 | 0·01 | <0·3 | — |
| (*c*) | 4 | 0·01—0·03 | <0·1 | <0·01 | <0·01 | 0·1—0·3 | — |
| | 5 | 0·03—0·1 | <0·1 | <0·01 | 0·01—0·03 | 0·3—1·0 | — |
| | 7 | — | — | — | — | — | 1·0 mg. |
| (*d*) | 2 | 0·03—0·1 | 0·1 | 0·01 | 0·01—0·03 | 0·3—1·0 | — |
| | 3 | 0·1 —0·3 | 0·1 | 0·01 | 0·01—0·03 | 0·1—0·3 | 0·8 mg. |
| | 4 | — | — | — | — | — | 0·6 ,, |
| | 5 | — | — | — | — | — | 1·1 ,, |

*Note.*—Tungsten was determined by the standard colorimetric thiocyanate procedure.

Results obtained in two experiments showed that in one case, where niobium only was present in the sample, niobium was partly extracted in the last 200 ml. of the solvent containing 1% of hydrofluoric acid, and in the second case, where tantalum was present in addition to niobium, the same effect was again observed. It thus appeared that the presence of some foreign ions is necessary to prevent partial extraction of niobium with this solvent. However, this pheno-

menon was not considered to be serious since it was easy to collect the final 200 ml. of the low-acidity solvent and combine it with the high-acidity solvent containing the main niobium fraction. This procedure would, of course, be unnecessary when analysing samples containing appreciable amounts of foreign ions, particularly in a routine laboratory when similar samples are under analysis, since it is possible to check whether any niobium is extracted in the low-acidity solvent for one particular type of sample.

The results shown in Table 3 were obtained by the new chromatographic procedure for pure Ta–Nb mixtures.

TABLE 3.

*Solvents employed*: Ethyl methyl ketone (*a*) saturated with water, (*b*) containing 1% of hydrofluoric acid, (*c*) containing 12·5% of hydrofluoric acid.

| Taken | | | Nb, mg. | | | |
|---|---|---|---|---|---|---|
| Ta, mg. | Nb, mg. | Ta found, mg., in 250 ml. of solvent (*a*) | 1st 200 ml. of solvent (*b*) | 2nd 200 ml. of solvent (*b*) | 400 ml. of solvent (*c*) | Total Nb, mg. |
| nil | 172·8 | 1·0 (blank) | — | 14·4 | 158·9 | 173·2 |
| nil | 200·2 | 0·5 | 1·0 | 1·0 | 199·0 | 201·0 |
| 79·6 | 212·2 | 80·9 | — | 10·5 | 201·3 | 211·8 |
| 17·6 | 211·0 | 18·5 | 1·0 | 25·8 | 185·7 | 212·5 |

*Spectrographic analysis*: All tantalum fractions contained <0·1% of Nb; all niobium fractions contained < 0·1% of Ta.

The results shown in Table 4 were obtained for samples of various synthetic concentrates to which tantalum and niobium had been added. Presumably because of the presence of foreign ions, the low-acidity solvent did not cause extraction of niobium in amount greater than about 2 mg.

TABLE 4.

Concentrate I: equal amounts of $TiO_2$, $SnO_2$, $ZrO_2$, $Fe_2O_3$, $ThO_2$, $Al_2O_3$, $SiO_2$, and Ce and lanthanons.
Concentrate V: $TiO_2$, 30; $SnO_2$, 15; $ZrO_2$, 10; $MnO_2$, 5; $Fe_2O_3$, 5; $WO_3$, 10 parts.

| | Taken, mg. | | Concentrate | | Ta found, | Nb found, mg. | | Total Nb, |
|---|---|---|---|---|---|---|---|---|
| Expt. no. | Ta | Nb | taken, | g. | mg. | Solvent (*b*) | Solvent (*c*) | mg. |
| 1 | — | 21·5 | V | 0·113 | <1·0 | <1·0 | 22·5 | 22·5 |
| 2 | 107·2 | 202·4 | I | 0·25 | 107·3 | 2·2 | 199·9 | 202·1 |
| 3 | 11·6 | 20·3 | I | 1·0 | 12·1 | <1·0 | (Not determined) | |
| 4 | 4·4 | 20·6 | I | 1·0 | 4·6 | <1·0 | 20·5 | 20·5 |
| 5 | 44·7 | 208·7 | $TiO_2$ | 0·074 | 47·0 | 2·3 | 203·0 | 205·3 |

*Notes on Results.*—Expt. (1). The excess of niobium found over that added was shown to be due to tungsten. Spectrographic analysis indicated that all other impurities were less than 1 mg.
Expt. (2). Both the separated Ta and Nb contained less than 1 mg. of impurities. The last 200 ml. of the intermediate solvent (*b*) contained 2·2 mg. of Nb.
Expt. (3). The Ta and Nb fractions were spectrographically pure.
Expt. (4). The Nb contained <1 mg. of impurity.
Expt. (5). The Ta and Nb fractions were free from titanium.

The results show that tantalum and niobium can be directly determined by the chromatographic procedure, in the presence of comparatively large amounts of other ions including titanium, tin, and zirconium. Any tendency for niobium to be extracted by the intermediate solvent is overcome by collecting the last 200 ml. of this solvent, which is then combined with the main niobium fraction.

(4) *Chemical Treatment of Samples before Chromatographic Separation of Tantalum and Niobium, and its Comparison with the Direct Chromatographic Procedure.*—During the analysis of a number of samples of columbite and ferro–tantalum–niobium alloys it was found that direct application of the chromatographic procedure as described above gave slightly low results owing to small hold-ups of tantalum and sometimes niobium in the column (*J.*, 1952, 1497). Further experiments indicated that this was due to the presence of foreign ions, some of which, depending upon the amount present, may be more troublesome than others. The difficulty has been overcome by making a simple chemical separation, before chromatography (see p. 3162); in this way the bulk of metals such as iron, cobalt, nickel, etc., are easily removed. In most cases, in routine work, this separation is probably unnecessary.

The chemical separation, based in part on the procedure described by Bagshawe and Elwell (*J. Soc. Chem. Ind.*, 1947, **66**, 398), involves separation of the earth acids in dilute hydrochloric acid. Depending upon the amount of earth acids present and also the grade and type of sample,

one or sometimes two separations were made. The major portion of tantalum and niobium were precipitated in hydrochloric acid containing 10% (v/v) of acid of *d* 1·18, which also ensures solubility of part of the titanium, tin, and zirconium and effects almost complete separation of common ions such as Fe. The residual earth acids were then precipitated after the acid concentration in the filtrate had been reduced to 2%. The combined earth-acid precipitate was ignited, and the oxides treated as a sample for the chromatographic determination of tantalum and niobium (see p. 3162).

Typical results obtained by this double precipitation procedure for synthetic samples are given in Table 5. In Table 6 results obtained by the direct chromatographic procedure after chemical separation are compared for a number of standard types of sample (including columbite and ferro–tantalum–niobium alloys).

The results shown in Tables 5 and 6 indicate the general usefulness of the chomatographic procedure for the determination of tantalum and niobium in complex materials. In most cases a direct determination without chemical separation is sufficiently accurate, particularly for medium-grade samples. In such cases the intermediate solvent can usually be discarded. When, however, a very accurate result is required, and particularly with high-tantalum–niobium samples, it is preferable to make the simple chemical separation before chromatography. In this way the bulk of ions such as iron, which may cause a small hold-up of earth acids

TABLE 5.

Concentrate I : See Table 4.
Concentrate III : Equal amounts of $Al_2O_3$, $ThO_2$, $Fe_2O_3$, lanthanons, $SiO_2$.
Concentrate IV : ,, ,, $CaF_2$, $WO_3$, MgO, PbO, $MnO_2$.
Concentrate VI : ,, ,, $ThO_2$, $Al_2O_3$, $CaF_2$, $SiO_2$, lanthanons ; 25 parts of this mixture together with $Fe_2O_3$, 10 ; MgO, 3 ; PbO, 2 ; $MnO_2$, 2 parts.

| Expt. no. | Taken, mg. Ta | Nb | Concentrate No. and wt., g. | | Ta found, mg. | Nb oxides found, mg. | Nb found, mg. |
|---|---|---|---|---|---|---|---|
| 1 | nil | 207·6 | I<br>III<br>IV | 0·17<br>0·77<br>0·11 | <1·5 | 296·9 | 205·0 |
| 2 | 51·8 | 151·2 | VI | 1·0 | 51·4 | — | 149·0 |
| 3 | 95·0 | 192·8 | I | 1·0 | 93·2 | — | 193·2 |

*Notes.*—$Ta_2O_5$ : In all cases the impurities were negligible and <0·1% of Nb was present.
$Nb_2O_5$ : From Expt. (1). Ta, <0·1 ; Fe, 0·03—0·1 ; Sn, 0·01—0·03 ; Ti, <0·1 ; Zr, <0·3%. (The weight of niobium in the last column has been corrected for tungsten, since the oxides contained 5 mg. of $WO_3$.) From Expt. (2). All impurities <0·1%. From Expt. (3). The $Nb_2O_5$ contained 1 mg. of $TiO_2$ as impurity. The last 200 ml. of the intermediate solvent contained 19·3 mg. of oxides ; this was combined with the main niobium fraction which was free from titanium.

TABLE 6. *Volume of solvent used for extraction of niobium (ethyl methyl ketone containing 12·5% of hydrofluoric acid).*

In Expts. 1 and 2, 400 ml. of solvent were taken, but in Expt. 3, 500 ml. were required.

| Expt. no. | Type of sample and weight taken : Type | G. | Type of determination | Found, % $Ta_2O_5$ | Ta | Found, % $Nb_2O_5$ | Nb |
|---|---|---|---|---|---|---|---|
| 1 | Pyrochlore | 0·6246 | Direct | 3·01 | — | 36·66 | — |
| 2 | Columbite | 0·4585 | ,, | 29·85 | — | 36·75 | — corr. for W |
| 3 | ,, | 0·5047 | Initial chemical sepn. | 30·61 | — | 36·60 | — |
| 4 | Ferro–Ta–Nb (Standard sample) | 0·4577 | Direct | — | 13·03 | — | 51·85 (corr. for W, 51·20) |
| 5 | ,, | 0·4373 | Initial chemical sepn. | — | 13·27 | — | 52·30 (corr. for W, 52·0) |
| 6 | Ferro–Ta–Nb | 0·3004 | Direct | — | 5·74 | — | 60·60 (corr. for W, 60·02) |
| 7 | ,, | 0·4616 | Initial chemical sepn. | — | 5·85 | — | 61·26 (corr. for W, 60·76) |
| 8 | Steel (Cr, 18; Ni, 8; Mo, 3%) | 4·0 g. | ,, ,, | — | <0·05 | — | 0·85 |
| 9 | Steel (Cr, 18; Ni, 8%) | 4·0 g. | ,, ,, | — | <0·05 | — | 0·91 |

For notes see p. 3161.

*Notes.*—Expt. 1. Total impurities in both oxides were less than 0·1%. The same pyrochlore, when analysed by the original technique (*J.*, 1952, 1504), gave niobium oxide which contained 5% of titanium oxide.

Expt. 2. Tungsten extracted with the niobium was equivalent to 0·37% ($WO_3$) on the original sample; otherwise the oxides were spectrographically pure.

Expt. 3. Apart from tungsten in the extracted niobium, the oxides were spectrographically pure. The last 200 ml. of the intermediate solvent contained 2·0 mg. of oxides.

Expt. 4. The niobium oxide contained 1·25% of $WO_3$; other impurities were absent. The last 200 ml. of the intermediate solvent contained 3·1 mg. of oxides.

Expt. 5. The niobium oxide contained 0·56% of $WO_3$ but no other impurity.

Expt. 6. ,, ,, 0·96% of $WO_3$ ,, ,,

Expt. 7. ,, ,, 0·82% of $WO_3$ ,, ,, The last 200 ml. of the intermediate solvent contained 1·0 mg. of oxides. At least 500 ml. of the solvent containing 12·5% of hydrofluoric acid were required for extraction of niobium; the final 100 ml. gave 7·8 mg. of oxides.

Expt. 8. The chemical pre-treatment employed in the analysis of this sample was shorter than that applied in Expts. 1—7 in that only one precipitation of the earth acids was made in a volume of 250 ml. at an acidity of 2% of hydrochloric acid (*J. Soc. Chem. Ind.*, 1947, **66**, 398) (see p. 3163). A sample weight of 4 g. was used. Owing to the presence of molybdenum in the sample extracted by the solvent used for niobium, the niobium was determined by a precipitation procedure employing ammonia, before ignition to oxide. This procedure has also been used for samples containing phosphorus and will be described more fully in a later paper. The niobium oxide from this analysis contained less than 1% of impurity.

Expt. 9. The pre-chemical separation employed was similar to that used in Expt. 8; niobium was determined by direct ignition, since molybdenum and phosphorus were absent from the original sample.

(especially tantalum) in the column, are removed at the outset. These effects are well illustrated by Expts. 2 and 3, and 4 and 5 (Table 6). When the sample contains less than about 2% of earth acids, it is usually preferable to make a chemical separation (see Expts. 8 and 9), otherwise the large sample which may be required would be extremely difficult to handle on the column.

Experience has shown that a sample weight should be chosen such that not more than about 175—200 mg. of each earth-acid element is present, otherwise the capacity of the cellulose may be exceeded. In general, it has been found that these quantitites of the metals are easily extracted in the volumes of solvents recommended, *i.e.*, 250 ml. of water-saturated solvent for tantalum and 400—500 ml. of the ethyl methyl ketone–12·5% hydrofluoric acid solvent for niobium.

*Method for the Direct Chromatographic Determination of Tantalum and Niobium in their Mixtures with Other Metal Ions* (*including* Ti, Sn, Zr, Th, *lanthanons*, Fe, Al, Ca, Mg, Pb, Ce, etc.).

*Solvents.—Ethyl methyl ketone.* The commercial product was purified as described in Part XI (*J.*, 1952, 1501).

*Solvent A* (for extraction of tantalum). This was prepared as already described (*ibid.*).

*Solvent B* (intermediate solvent). 1 Ml. of hydrofluoric acid solution (40% w/w) was added to each 99 ml. of ethyl methyl ketone.

*Solvent C* (for extraction of niobium). 12·5 Ml. of hydrofluoric acid solution (40% w/w) were added to each 87·5 ml. of ethyl methyl ketone.

*Decomposition of Sample and its Preparation for Chromatography.*—Samples (pyrochlore, columbite, ferro–tantalum–niobium, etc.) were decomposed by treatment with hydrofluoric and nitric acid mixtures in large platinum dishes (3" diam.), and were finally converted into fluorides by repeated evaporation with hydrofluoric acid. Decomposition and evaporations were effected on the steam-bath (see also *J.*, 1952, 1497). 8 Ml. of 25% hydrofluoric acid (40% w/w) were added to the dry residue, and the whole was gently warmed under a platinum cover for about 10 minutes to effect solution. Evaporation was carefully avoided during this operation. Ammonium fluoride (1 g.) was then added, and heating was continued for a few more minutes to effect solution. The final solution was allowed to cool, and 6 g. of cellulose (Whatman standard grade) were then added, and the whole well mixed with a short Polythene rod to form a homogeneous friable mass.

*Chromatographic Extraction.* A column of activated cellulose was prepared and conditioned with solvent A as already described (*J.*, 1952, 1501). The sample, absorbed on cellulose, was then transferred to the top of the column and the empty dish was allowed to rest in the funnel. Solvent was always poured into the dish with stirring, before transfer to the column. Solvent A was added by this method, in sufficient quantity to reach a level of about 2 cm. above the cellulose wad, which was then agitated by means of a Polythene plunger so that it formed a homogeneous continuation to the main column. The plunger was washed with solvent A, sufficient being

used to reach nearly to the top of the tube (to funnel-join). When this first batch of solvent A had reached the level of the cellulose, the addition of solvent was continued until 250 ml. had been used. The eluant containing the tantalum was collected in a 600-ml. Polythene beaker, which was replaced by a similar beaker when the last of solvent A had fallen to the level of the cellulose. Solvent B was next poured into the column *via* the platinum dish, so that it came about half-way to the level of the top of the column tube. When this solvent had fallen to the level of the cellulose, a further similar quantity was added, and the sample wad gently beaten with the plunger, and the extraction continued in the manner described above until 400 ml. of solvent B had been used. Depending on the type of sample under analysis, the last 200 ml. of solvent B could contain some niobium. This possibility is readily established for a particular type of ore by one determination in which the appropriate fraction of solvent B is analysed for niobium. With analyses on medium-grade ores containing appreciable amounts of impurities, the whole of this intermediate solvent fraction could normally be discarded. In the analysis of very high-grade ores or samples where a pre-chemical separation had been made (see below) it was often necessary to collect the last 200 ml. of solvent B and combine it with the main niobium fraction (solvent C).

When the last of solvent B had fallen to the top of the " wad " the extraction was continued in the manner described, with solvent C (in this case the wad was not agitated), 400 ml. being collected for low-grade samples or 500 ml. for medium- or high-grade samples. This fraction contained niobium and if necessary was combined with the last 200 ml. of solvent B.

*Removal of Solvents and Determination of Tantalum and Niobium.*—The solvent was removed from each of the fractions in the manner already described (*J.*, 1952, 1502) by evaporation in a stream of air. The aqueous residue was then transferred to a weighed platinum dish (diam. about 2") by means of a fine jet of water and a rubber-tipped rod. Sulphuric acid (2 drops of 50% solution) was added, and the solution gently evaporated to a volume of about 1 ml. under infra-red lamps. Sufficient ashless paper was added to absorb the solution, and the dish was carefully ignited, finally to about 900°. The resulting oxide was then weighed ($Ta_2O_5$ or $Nb_2O_5$). This direct ignition procedure was found to be quicker and preferable to the tannin procedure (*J.*, 1952, 1502).

*Purity of Tantalum and Niobium Oxides.*—Provided that phosphorus and molybdenum were absent from the original sample, the only impurity for which a correction was necessary was tungsten in the niobium oxide. This metal was determined by the colorimetric thiocyanate procedure, carried out in strongly acid solution (HCl) after fusion of the oxide with sodium carbonate.

*Method with Chemical Pre-treatment of the Sample, followed by Chromatographic Analysis.*

When a high degree of accuracy was required, or when a large amount of sample was needed such as with low-grade materials, most of the common metal ions were first removed by precipitation of the earth acids from an acid solution. Since titanium, tin, and zirconium are very strongly absorbed by earth acids, unless the concentration of the earth acids was very low, this chemical treatment only brought about a small reduction in these ions.

Medium- or high-grade samples (0·3—0·5 g.) were decomposed in the usual manner with hydrofluoric and nitric acids in a large platinum dish (see p. 3160), and after final evaporation to dryness, 20 ml. sulphuric acid solution (50% v/v) were added, and the contents of the dish taken to strong fumes of sulphuric acid under infra-red lamps and then allowed to fume for 1 hour to remove fluoride ions. After cooling, the contents of the dish were washed into a 1-l. beaker, a total of about 100 ml. of being water used. After addition of 50 ml. of hydrochloric acid (*d* 1·18) and 30 ml. of hydrogen perioxde (100-vol.), the beaker was covered with a clock-glass and gently warmed for a short period to assist solution of earth acids and titania. The solution was diluted to about 600 ml. and boiled for 30 minutes; this usually gave a clear solution which gradually deposited earth acids and adsorbed impurities such as $TiO_2$, $SnO_2$, $WO_3$. After cooling, the precipitate was collected on a No. 541 Whatman paper, washed with dilute hydrochloric acid (1% solution), and reserved for further treatment. The filtrate was returned to the original beaker and neutralised by addition of ammonia (*d* 0·88). After addition of 10 ml. of hydrochloric acid (*d* 1·18) and about 50 ml. of a saturated solution sulphur dioxide, the volume was made up to about 600 ml., and the solution then boiled for about 20 minutes and set aside for about 10 hours or overnight. The precipitate was filtered on a No. 541 Whatman paper, washed, and then combined with the main earth-acid precipitate in the original platinum dish. It was then ignited, and the oxides treated as a sample for chromatographic analysis. Tantalum and niobium were determined as described in the first procedure (p. 3161). Depending on the type of

sample, it was sometimes necessary to combine part of the intermediate solvent fraction with the main niobium fraction.

*Low-grade Materials.*—The blank values obtained in the direct procedure on 1-g. samples of mixed ions (Fe, Al, Ti, Zr, etc.) were usually of the order of up to 1·5 mg. (oxide) for tantalum and 2 mg. (oxide) for niobium when the earth acids were absent, so in the analysis of low-grade samples comparatively large amounts (5 g.) were often needed. Such samples were too large for direct chromatographic analysis, so it was always necessary to make a previous chemical separation. The procedure adopted (*e.g.*, the analysis of steels) was that described by Bagshawe *et al.* (*loc. cit.*) which was shorter than that described above since only one precipitation was involved. The procedure adopted for samples of steel involved decomposition of the sample in hydrochloric acid in a 500-ml. beaker, evaporation to dryness, and baking for about 1 hour under infra-red lamps. The residue was moistened with 5 ml. of hydrochloric acid (*d* 1·18), followed by addition of about 20 ml. of water, and the whole then warmed to effect solution. After dilution to 250 ml., about 30 ml. of a saturated solution of sulphur dioxide were added to reduce ferric iron, and the solution boiled for about 20 minutes and then kept overnight. After filtration, the earth-acid precipitate was ignited, and the tantalum and niobium determined chromatographically. The whole of the intermediate solvent fraction could usually be discarded.

Samples which contained large amounts of silica were treated with hydrofluoric acid in order to remove the silica at the outset of the analysis.

The analysis of low-grade samples, particularly those containing phosphorus, will be considered more fully in a future publication.

The author thanks Dr. R. B. F. Temple and Mr. J. R. Moore, who carried out the spectrographic work, and Mr. R. A. Mercer and Miss Patricia Swain for experimental assistance.

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Radiochemical Group, Chemical Research Laboratory,
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## 604. *Eight- and Higher-membered Ring Compounds. Part X.* Di-, Tri-, and Tetra-thiosalicylides.*

By Wilson Baker, A. S. El-Nawawy, and W. D. Ollis.

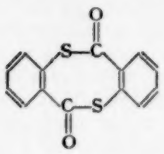
Dehydration of thiosalicylic acid gives dithiosalicylide and the hitherto unknown tri- and tetra-thiosalicylides. Alkaline hydrolysis of these compounds regenerates thiosalicylic acid. Di- and tri-thiosalicylides react with benzylamine in boiling benzene to give *N*-benzylthiosalicylamide, which by desulphurisation with Raney nickel gave *N*-benzylbenzamide. When heated with benzylamine an unexpected reaction occurs, the three thiosalicylides each giving *NS*-dibenzylthiosalicylamide.

" Dibenzthiophthene " is shown to be 4′ : 5′-4 : 5-dibenzothiopheno(2′ : 3′-3 : 2)thiophen (VII), because desulphurisation gives dibenzyl and 1 : 2 : 3 : 4-tetraphenyl*cyclo*butane.

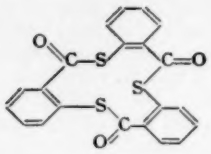
The many-membered cyclic anhydro-derivatives of salicylic acid, of three of the cresotic acids, and of thymotic acid, were the subjects of investigations described in Parts II, III, and VI of this series (Baker, Ollis, and Zealley, *J.*, 1951, 201; Baker, Gilbert, Ollis, and Zealley, *J.*, 1951, 209; Baker, Gilbert, and Ollis, *J.*, 1952, 1443). From each of these acids *cis*-di-(8-membered), tri-(12-membered), and tetra-(16-membered)-anhydro-derivatives have been prepared, and in addition, salicylic acid and probably *m*-cresotic acid yielded hexa-(24-membered)-anhydro-compounds. The enquiry has now been extended to thiosalicylic acid, from which only di(thiosalicylide) (I) has previously been

* Part IX, *J.*, 1952, 2991.

prepared (R. Anschütz and Rhodius, *Ber.*, 1914, **47**, 2733), and we find that dehydration can also lead to tri(thiosalicylide) (II), and tetra(thiosalicylide) (III).



(I)



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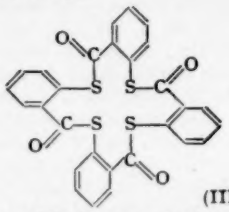


(III)

Although the distillation *in vacuo* of *O*-acetylsalicylic acid gives good yields of *cis*-di- and tri-salicylide (see Part II), the distillation of *S*-acetylthiosalicylic acid gives, according to Anschütz and Rhodius (*loc. cit.*) only a di(thiosalicylide) (I), accompanied by diphenyl disulphide, " dibenzthiophthene," and 9-thioxanthone (the yields of these products are not stated). Repetition of this experiment gave di(thiosalicylide) (5%), diphenyl disulphide (40%), " dibenzthiophthene " (3%) (its structure is discussed below), and traces of 9-thioxanthone and of tetra(thiosalicylide) (III). It has been shown previously that dehydration of salicylic acid yields disalicylide only under mild conditions, and gives increasing amounts of higher salicylides when more vigorous methods are used; these results are in contrast with those obtained by dehydrating thiosalicylic acid, which gives di(thiosalicylide) only under vigorous conditions.

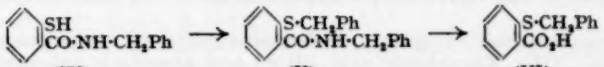
Dehydration of thiosalicylic acid with phosphoric anhydride in toluene or xylene gave di(thiosalicylide) (I) (14—32%), tri(thiosalicylide) (II) (0—15%), and tetra(thiosalicylide) (III) (0—20%) in varying proportions according to the experimental conditions. Increase in the reaction temperature or in the amount of phosphoric anhydride favours the formation of di(thiosalicylide) over the tri- and tetra-compounds; this is contrary to the behaviour of salicylic acid which, under the same conditions, gave the tetramer and hexamer, as well as traces of the trimer. Thiosalicylic acid reacts with phosphorus oxychloride in xylene to give di- and tri-(thiosalicylide) in poor yield. With trifluoroacetic anhydride in xylene thiosalicylic acid gives very small amounts of di-, tri-, and tetra-(thiosalicylide); salicylic acid under these conditions gives small amounts of tetra- and hexa-salicylide.

Di-, tri-, and tetra-(thiosalicylide) are readily hydrolysed to thiosalicylic acid by 5% ethanolic sodium hydroxide, but are relatively stable towards acid hydrolysis, and they are unchanged by phosphorus oxychloride in boiling xylene (cf. conversion of di- and tri-salicylide into tetra- and hexa-salicylides under these conditions; Part II, *loc. cit.*). Both di- and tri-(thiosalicylide) separate from benzene with solvent of crystallisation which is not present in stoicheiometric amount; these complexes are presumably clathrates (cf. tetra-*m*- and tri-*p*-cresotide, Part III, and tri-*o*-thymotide, Part VI).

The dipole moment of di(thiosalicylide) (6·39 D) shows that it possesses the *cis* (or " trough ") rather than the centro-symmetrical *trans* (or " chair ") constellation (cf. *cis*-disalicylide, the *cis*-dicresotides, and *cis*-dithymotide, which have dipole moments varying from 6·26 to 6·74 D; Edgerley and Sutton, *J.*, 1951, 1069, and unpublished observation by Dr. L. E. Sutton and Mr. M. F. Saxby, Oxford—see Part VI, *loc. cit.*). The dipole moment of tri(thiosalicylide) (1·54 $\pm$ 0·02 D) shows that it probably has a constellation similar to that proposed for trisalicylide (Edgerley and Sutton, *loc. cit.*).

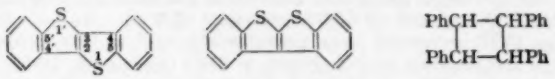
Di- and tri-(thiosalicylide) react normally with benzylamine in boiling benzene to give *N*-benzylthiosalicylamide (IV), and this amide is reduced by Raney nickel to *N*-benzylbenzamide. All three thiosalicylides, however, react abnormally with benzylamine at the boiling point giving *NS*-dibenzylthiosalicylamide (V), the structure of which follows from the facts that (*a*) alkaline hydrolysis gives *S*-benzylthiosalicylic acid (VI), (*b*) desulphurisation with Raney nickel gives *N*-benzylbenzamide and some dibenzyl, (*c*) it may be synthesised by benzylation of *N*-benzylthiosalicylamide (IV) by benzyl chloride and potassium carbonate in acetone or by boiling benzylamine alone. The amide (IV) is clearly an intermediate in the formation of (V) from the thiosalicylides. When boiled with benzylamine, thiosalicylic acid gave a mixture of (IV) and (V).

" ***Dibenzthiophthene.***"—This substance, $C_{14}H_8S_2$, isolated in small (3%) yield from the products of distillation of *S*-acetylthiosalicylic acid (Anschütz and Rhodius, *loc. cit.*), is obtained in 16% yield along with di(thiosalicylide) by heating thiosalicylic acid with




(IV) (V) (VI)

phosphoric anhydride in tetralin. Anschütz and Rhodius suggested two formulæ for this compound, *viz.*: (VII) and (VIII); and (VII) is now shown to be correct by desulphurisation with Raney nickel. With a freshly prepared specimen of Raney nickel in ethanol it gave 1 : 2 : 3 : 4-tetraphenyl*cyclo*butane (IX) but with Raney nickel which had been kept




(VII) (VIII) (IX)

for three months it gave a mixture of tetraphenyl*cyclo*butane and dibenzyl. When methanol was used as solvent, Raney nickel, either freshly prepared or aged, gave dibenzyl. Both results prove that the benzene rings are united by a $C_2$ bridge, thus excluding structure (VIII), and it may be observed that only 4′ : 5′-4 : 5-dibenzothiopheno(2′ : 3′-3 : 2)thiophen (VII) contains the carbon skeleton of *two* thiosalicylic acid units. A diradical, ·CHPh–CHPh· is probably involved in the formation of tetraphenyl*cyclo*butane and of dibenzyl by the desulphurisation of (VII) (see Kenner, Lythgoe, and Todd, *J.*, 1948, 961). Stilbene is not an intermediate in the reaction of (VII) with Raney nickel, as it gives neither dibenzyl nor 1 : 2 : 3 : 4-tetraphenyl*cyclo*butane under these conditions.

## Experimental

M. p.s are uncorrected. Molecular weights were determined with a Menzies–Wright apparatus as described in Part II (*J.*, 1951, 208). Analyses are by Drs. Weiler and Strauss, Oxford, and by Mr. W. M. Eno, Bristol.

*Dehydration of Thiosalicylic Acid.*—(*a*) *Phosphoric anhydride in toluene.* cis-*Di*(*thiosalicylide*) (I), *tri*(*thiosalicylide*) (II), *and tetra*(*thiosalicylide*) (III). Thiosalicylic acid (15 g.) and phosphoric anhydride (45 g.) were heated under reflux in dry toluene (200 c.c.) for 5·5 hours; after cooling, the solid cake on the surface was separated and triturated with hot chloroform. The combined toluene and chloroform solutions were washed with aqueous 5% sodium hydrogen carbonate, dried, and evaporated under diminished pressure. The residue was fractionally crystallised from benzene, and the two components, which both contain benzene of crystallisation, separated by handpicking; this is facilitated by the fact that di(thiosalicylide) rapidly loses its benzene of crystallisation at room temperature and the crystals become opaque. The *cis*-di(thiosalicylide) (II) (1·9 g., 14%) was recrystallised from benzene, giving the *benzene* complex as large, thick parallelograms (Found: loss in wt. when a freshly prepared specimen was kept at 100°, 13·5%. $C_{14}H_8O_2S_2,0{\cdot}55C_6H_6$ requires $C_6H_6$, 13·6%).

Crystallisation from chloroform gave unsolvated *cis*-di(thiosalicylide) as irregular, thick rhombs, m. p. 176—177° [Anschütz and Rhodius, *loc. cit.*, gave m. p. 175° (rapid heating); the two m. p.s mentioned by them were not verified] (Found: C, 61·3; H, 3·1; S, 23·25%; *M*, ebullioscopic in benzene, 293; *M*, ebullioscopic in chloroform, 282. Calc. for $C_{14}H_8O_2S_2$: C, 61·7; H, 2·9; S, 23·5%; *M*, 272).

The *tri*(*thiosalicylide*) (II) (2·0 g., 15%) separated from benzene in long, colourless prisms containing solvent of crystallisation (Found: loss in wt. at 100° of a freshly prepared specimen, 12·2%. $C_{21}H_{12}O_3S_3,0{\cdot}725C_6H_6$ requires $C_6H_6$, 12·2%). Crystallisation from chloroform or dioxan gave unsolvated tri(thiosalicylide), m. p. 257—258° (Found: C, 61·8; H, 2·7; S, 23·7%; *M*, ebullioscopic in benzene, 416; *M*, ebullioscopic in chloroform, 420. $C_{21}H_{12}O_3S_3$ requires C, 61·7; H, 2·9; S, 23·5%; *M*, 408).

The solid (3·1 g.; m. p. 248—250°) which separated from the cooled reaction mixture and was insoluble in hot chloroform (see above) was crystallised several times from dioxan–chloroform and finally from dioxan, giving *tetra*(*thiosalicylide*) (2·6 g., 20%) as long needles, m. p.

288—290° (rapid heating) (Found: C, 61·7; H, 3·7; S, 22·9%; *M*, ebullioscopic in benzene, 556; *M*, ebullioscopic in chloroform, 554. $C_{24}H_{16}O_4S_4$ requires C, 61·7; H, 2·9; S, 23·5%; *M*, 544). Small amounts of tri(thiosalicylide) were also isolated during these crystallisations. Tetra(thiosalicylide) is only slightly soluble in benzene but is very easily soluble in dioxan and chloroform.

(*b*) *Phosphoric anhydride in xylene.* cis-*Di*(*thiosalicylide*) (I), *tri*(*thiosalicylide*) (II), *and tetra*(*thiosalicylide*) (III). Thiosalicylic acid (20 g.) and phosphoric anhydride (30 g.) were boiled in xylene (250 c.c.) for 4 hours and worked up as in the previous case. The non-acidic product (6·9 g.) was extracted with hot benzene (150 c.c.), and the residue was dissolved in hot dioxan. The benzene extract gave *cis*-di(thiosalicylide) (3·1 g.; m. p. 176—177°) and tri(thiosalicylide) (1·5 g.; m. p. 257—258°), and the dioxan solution yielded tetra(thiosalicylide) (0·2 g.; m. p. 288—290°).

With 2½ times the amount of phosphoric anhydride and 24 hours' heating the products were "dibenzthiophthene" (0·3 g.) and *cis*-di(thiosalicylide) (2·2 g.). 4 : 5-4′ : 5′-*Dibenzothiopheno*-(2′ : 3′-3 : 2)*thiophen* (VII) separated from glacial acetic acid as thin, colourless flakes, m. p. 209—210° (Anschütz and Rhodius, *loc. cit.*, give m. p. 216°) (Found: C, 70·5; H, 3·6; S, 26·9%; *M*, ebullioscopic in benzene, 237. $C_{14}H_8S_2$ requires C, 70·0; H, 3·3; S, 26·7%; *M*, 240).

Similarly salicylic acid (35 g.), phosphoric anhydride (40 g.), and xylene (250 c.c.) gave, after 2·5 hours' heating, trisalicylide, m. p. 200° (0·5 g., 2%), tetrasalicylide, m. p. 298—300° (5·5 g., 20%), hexasalicylide, m. p. 375° (rapid heating) (3 g., 11%), and unchanged salicylic acid (4 g.).

*p*-Cresotic acid (10 g.) and phosphoric anhydride (15 g.) in xylene (200 c.c.) gave similarly tri-*p*-cresotide, m. p. 245° (0·9 g.), and tetra-*p*-cresotide, m. p. 347° (decomp.) (2·9 g.).

(*c*) *Phosphoric anhydride in tetralin.* cis-*Di*(*thiosalicylide*) *and dibenzothiophenothiophen.* Thiosalicylic acid (25 g.), boiled with phosphoric anhydride (40 g.) and tetralin (150 c.c.) for 3 hours, gave dibenzothiophenothiophen (3·4 g.) and *cis*-di(thiosalicylide) (4·2 g.); no other thiosalicylide was isolated.

(*d*) *Phosphorus oxychloride in xylene.* cis-*Di*(*thiosalicylide*), *tri*(*thiosalicylide*), *and dibenzothiophenothiophen.* Thiosalicylic acid (84 g.), redistilled phosphorus oxychloride (135 c.c.), and xylene (300 c.c.) were boiled for 8 hours, cooled, and poured into iced water (1·5 l.). The xylene layer, after being washed with aqueous 5% sodium hydrogen carbonate, finally gave a product which was fractionally crystallised from benzene, giving the less soluble dibenzothiophenothiophen (0·5 g.) and a mixture of *cis*-di- and tri-(thiosalicylide) which was separated as before. Recrystallisation gave *cis*-di(thiosalicylide) (2·6 g.; m. p. 176—177°) and tri(thiosalicylide) (0·6 g.; m. p. 257—258°).

(*e*) *Trifluoroacetic anhydride in xylene.* cis-*Di*(*thiosalicylide*), *tri*(*thiosalicylide*), *and tetra*-(*thiosalicylide*). Thiosalicylic acid (5 g.), trifluoroacetic anhydride (7 g.; Bourne, Stacey, Tatlow, and Tedder, *J.*, 1949, 2977), and xylene (150 c.c.) were boiled for 1·5 hours and worked up as in previous cases, giving *cis*-di(thiosalicylide) (0·3 g.), tri(thiosalicylide) (0·1 g.), and tetra(thiosalicylide) (0·1 g.); thiosalicylic acid (2·2 g.) was recovered.

Similarly salicylic acid (5 g.) and trifluoroacetic anhydride (7 g.) in xylene (50 c.c.) gave tetrasalicylide, m. p. 298—300° (15 mg.), and hexasalicylide (10 mg.); salicylic acid (1 g.) was recovered.

*Action of Heat on* S-*Acetylthiosalicylic Acid.* cis-*Di*(*thiosalicylide*, *Dibenzothiophenothiophen, and Diphenyl Disulphide* (cf. Anschütz and Rhodius, *loc. cit.*).—*S*-Acetylthiosalicylic acid (20 g.) was heated just above its m. p. (*ca.* 130°) at 22—26 mm. in the apparatus described by Baker, Ollis, and Zealley (Part II, *loc. cit.*) until no more acetic acid distilled. The temperature was raised to 160—170° and the yellow sublimate (7·5 g.) was dissolved in acetic acid. Dibenzothiophenothiophen (0·4 g.; m. p. 209—210°) separated and water was added to the mother-liquors, giving a precipitate (5·5 g.) which was fractionally crystallised from light petroleum (b. p. 40—60°). Recrystallisation of the various fractions from the appropriate solvent gave diphenyl disulphide (4·5 g.; m. p. 60—61°) (lit., m. p. 60°); *cis*-di(thiosalicylide) (0·76 g.; m. p. 176—177°), 9-thioxanthone, m. p. 202—203° (lit., m. p. 207°), and a trace of tetra(thiosalicylide), m. p. 282—284°.

*Alkaline Hydrolysis of the Thiosalicylides.*—The finely powdered thiosalicylide was boiled with excess of 5% ethanolic sodium hydroxide for 3 hours and evaporated under diminished pressure. Addition of dilute hydrochloric acid and extraction with ether gave thiosalicylic acid, m. p. and mixed m. p. 163—164°, in each case [yields from *cis*-di(thiosalicylide), 68%, tri(thiosalicylide), 70%, and tetra(thiosalicylide), 60%].

N-*Benzylthiosalicylamide* (IV) *and its Desulphurisation.*—Di- or tri-(thiosalicylide) (250 mg.)

and benzylamine (1 c.c.) in benzene (50 c.c.) were boiled in an atmosphere of nitrogen for 7 hours, the benzene evaporated under diminished pressure, and the residue triturated with dilute acid. The solid (350 mg., 79%) was recrystallised from ethanol–water, giving *N-benzylthiosalicylamide* (IV) as needles, m. p. 204—206° (Found: C, 69·1; H, 5·1; N, 5·8; S, 13·8. $C_{14}H_{13}ONS$ requires C, 69·1; H, 5·35; N, 5·8; S, 13·2%).

*N*-Benzylthiosalicylamide (0·5 g.) and Raney nickel (*ca.* 3 g.) in ethanol (80 c.c.) were boiled for 4 hours and filtered while hot through Filtercel, which was washed twice with hot ethanol (50 c.c.). The ethanol yielded a residue of *N*-benzylbenzamide (0·25 g.; m. p. 100°) which separated from aqueous ethanol as needles, m. p. and mixed m. p. with an authentic specimen, 103—104°.

NS-*Dibenzylthiosalicylamide* (V).—(*a*) *Reaction of the three thiosalicylides with excess of benzylamine.* Each thiosalicylide (0·5 g.), benzylamine (3 c.c.), and a trace of ammonium chloride were boiled for 3 hours, cooled, poured into water and made slightly acid. The resulting oil slowly solidified and was recrystallised from ether, giving NS-*dibenzylthiosalicylamide* (V) as needles, m. p. 97° (Found: C, 75·0; H, 5·75; N, 4·2; S, 9·6%; *M*, ebullioscopic in benzene, 336; *M*, ebullioscopic in chloroform, 317. $C_{21}H_{19}ONS$ requires C, 75·6; H, 5·7; N, 4·2; S, 9·6%; *M*, 333). The yields were *cis*-di- 91%, tri- 90%, and tetra-(thiosalicylide) 79%.

(*b*) *From N-benzylthiosalicylamide* (IV) *and benzyl chloride.* The amide (175 mg.), benzyl chloride (1·3 g.), anhydrous potassium carbonate (0·4 g.), and acetone (40 c.c.) were boiled for 2¾ hours, cooled, filtered, and evaporated under diminished pressure. Water was added and chloroform-extraction yielded a solid which was recrystallised from aqueous methanol, giving *NS*-dibenzylsalicylamide (190 mg.), m. p. and mixed m. p. 97—98°.

(*c*) *From N-benzylthiosalicylamide* (IV) *and benzylamine.* The amide (250 mg.) and benzylamine (5 c.c.) were boiled for 15 hours, cooled, poured into dilute acid, and extracted with chloroform. This extract yielded *NS*-dibenzylthiosalicylamide (0·3 g.), m. p. and mixed m. p. 97—98° after crystallisation from aqueous methanol.

(*d*) *From thiosalicylic acid and benzylamine.* The acid (2·0 g.), benzylamine (10 c.c.), and a trace of ammonium chloride were boiled for 5 hours, then poured into dilute acid, and the precipitate was washed with warm ether. The ethereal washings yielded the less soluble *N*-benzylthiosalicylamide (1·0 g.), m. p. and mixed m. p. 204—206°, and *NS*-dibenzylthiosalicylamide (1·0 g.), m. p. and mixed m. p. 97°.

*Raney Nickel Desulphurisation and Alkaline Hydrolysis of* NS-*Dibenzylthiosalicylamide* (V).—The amide (2 g.), Raney nickel (5 g.), and ethanol (80 c.c.) were heated for 5 hours, and after filtration through Filtercel the filtrate yielded a residue (1·1 g.) which was recrystallised from aqueous methanol, giving *N*-benzylbenzamide, m. p. and mixed m. p. 103—104°. From the mother-liquors traces of dibenzyl, m. p. and mixed m. p. 50—51°, were isolated.

*NS*-Dibenzylthiosalicylamide (0·66 g.) and 10% ethanolic sodium hydroxide (10 c.c.) were boiled for 16 hours, and the ethanol was removed under diminished pressure. After addition of dilute acid the precipitate (0·48 g., 99%), m. p. 178—180°, was sublimed at 150°/13 mm., and recrystallised from ethanol, giving *S*-benzylthiosalicylic acid, m. p. 189° (Found: C, 69·0; H, 5·0; S, 13·0. Calc. for $C_{14}H_{12}O_2S$: C, 68·8; H, 4·9; S, 13·1%) (Apitzsch, *Ber.*, 1913, **46**, 3102, gives m. p. 189°).

*Raney Nickel Desulphurisation of Dibenzothiophenothiophen* (VII).—(*a*) Dibenzothiophenothiophen (0·06 g.) and Raney nickel (2·0 g.; Brown, *J. Soc. Chem. Ind.*, 1950, **69**, 355) in methanol (30 c.c.) were boiled for 3 hours and worked up as in previous cases. The product was dibenzyl (0·03 g., 66%), m. p. and mixed m. p. 50—51°.

(*b*) Dibenzothiophenothiophen (0·44 g.) and freshly prepared Raney nickel (8 g.) were boiled in ethanol (75 c.c.) for 3·5 hours. The crude product (0·26 g.) was recrystallised from aqueous methanol, giving 1 : 2 : 3 : 4-tetraphenyl*cyclo*butane (IX) (0·18 g., 55%), m. p. 162—163° (Pailer and Müller, *Monatsh.*, 1948, **79**, 615, give m. p. 164—165°). A similar experiment using dibenzothiophenothiophen (0·18 g.) and a specimen of Raney nickel prepared 3 months earlier gave 1 : 2 : 3 : 4-tetraphenyl*cyclo*butane (0·05 g.) and dibenzyl (0·045 g.).

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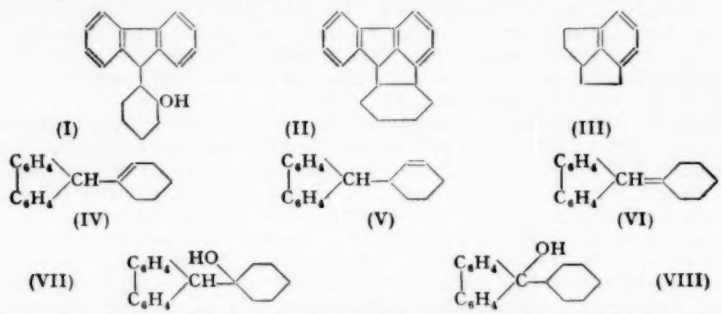
## 605. *Dehydration of 2-9′-Fluorenylcyclohexanol.*

By J. W. Cook and L. Hunter.

Catalytic hydrogenation of 2-9′-fluorenyl*cyclo*hexanone gave *cis*- and *trans*-2-9′-fluorenyl*cyclo*hexanol (I), both of which were dehydrated by phosphoric acid to 9-*cyclo*hex-1′-enylfluorene (IV), the structure of which has been established. This work extends and corrects that of Hurd and Mold (*J. Org. Chem.*, 1948, **13**, 339), who regarded the dehydration product as 1 : 9-*cyclo*hexylenefluorene (II).

By dehydration of 2-fluorenyl*cyclo*hexanol * (I) with syrupy phosphoric acid at 230°, Hurd and Mold (*J. Org. Chem.*, 1948, **13**, 339) obtained a hydrocarbon which they described as 1 : 9-*cyclo*hexylenefluorene (II). They supported their claim that *cyclo*dehydration had taken place by a statement that the hydrocarbon was inert towards bromine and towards potassium permanganate, and was therefore saturated. Its dehydrogenation to 9-phenylfluorene by heating it with palladium–charcoal or with selenium was attributed by Hurd and Mold to pyrolytic fission of a carbon–carbon bond.

The structure (II) seemed to us very improbable for the hydrocarbon in question, for it contains a highly strained ring system (III). von Braun and his collaborators (*Ber.*, 1917, **50**, 56; 1928, **61**, 956) have recorded unsuccessful attempts to prepare derivatives of (III) by internal Friedel–Crafts reactions (but cf. Koelsch, *J. Amer. Chem. Soc.*, 1932, **54**, 4746). It was suggested, therefore (Cook, *Nature*, 1949, **163**, 443), that the dehydration of 2-fluorenyl*cyclo*hexanol might be analogous to *cyclo*dehydration of 2-benzyl*cyclo*hexanol, and that the product is a derivative of the strainless 1 : 3 : 3-bi*cyclo*nonane.

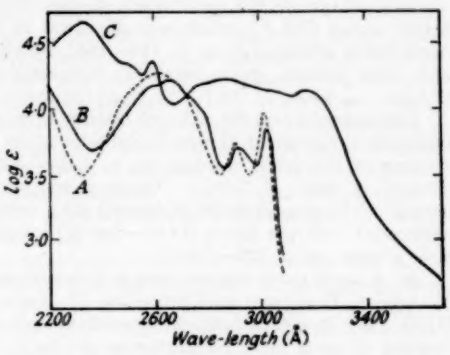



We have re-examined the dehydration of 2-fluorenyl*cyclo*hexanol under the conditions described by Hurd and Mold and have obtained a hydrocarbon having a m. p. (110·5—111°) in reasonable agreement with their figure (111·5—112°). This hydrocarbon was certainly not a product of *cyclo*dehydration for it was oxidised by chromic acid to fluorenone and not fluorenone-1-carboxylic acid. It was formed also by treatment of the alcohol with the milder dehydrating agent, potassium hydrogen sulphate, and was shown to be 9-*cyclo*hex-1′-enylfluorene (IV). On catalytic hydrogenation it was converted into the known 9-*cyclo*hexylfluorene. The presence of an ethylenic bond was confirmed by the formation of an epoxide with monoperphthalic acid, and of a diol with osmium tetroxide. The alternative structure of 9-*cyclo*hex-2′-enylfluorene (V) was made very improbable by the fact that the same hydrocarbon was obtained by dehydration of 1-fluorenyl*cyclo*hexanol (VII). Moreover, the possibility that double-bond migration had occurred to give *cyclo*hexylidenefluorene (VI) was excluded by the fact that the latter hydrocarbon (VI), prepared by dehydration of 9-*cyclo*hexylfluorenol (VIII), was distinct from the dehydration product of (I) and (II). The ultra-violet absorption spectra of the hydrocarbons (IV) and (VI) are in accord with the structures assigned to them. The Figure shows that 9-*cyclo*hex-1′-enylfluorene (IV) has a spectrum closely resembling that of fluorene, whereas the

* Throughout this paper, fluorenyl refers to the 9-fluorenyl radical.

spectrum of *cyclo*hexylidenefluorene (VI), in which the double bond is conjugated with both aromatic nuclei, is widely different. The spectrum of 9-*cyclo*hexylfluorene is almost indistinguishable from that of the *cyclo*hexenyl compound (IV).

For the preparation of 2-fluorenyl*cyclo*hexanol (I) we used Hurd and Mold's method (*loc. cit.*), namely, catalytic hydrogenation of 2-fluorenyl*cyclo*hexanone over copper chromite. This gave not only the alcohol, m. p. 123° (phenylurethane, m. p. 145°), which they described, but also a stereoisomeric alcohol, m. p. 127° (phenylurethane, m. p. 172°), and a hexahydride, m. p. 177—181°, of one of these. When the hydrogenation was carried out with a very active catalyst at a much lower temperature the sole product was the alcohol, m. p. 123°, which is therefore considered to be the *cis*-isomer. Both the *cis*- and the *trans*-alcohol were dehydrated by syrupy phosphoric acid to 9-*cyclo*hex-1′-enylfluorene (IV).




*A*, *Fluorene* (*after Ramart-Lucas*, Bull. Soc. chim., 1935, **2**, 1377).
*B*, 9-cyclo*Hex*-1′-*enylfluorene*.
*C*, 9-cyclo*Hexylidenefluorene*.

## Experimental

*Ethyl* 1-*Fluorenyl*-2-*keto*cyclo*hexane*-1-*carboxylate*.*—Hurd and Mold (*loc. cit.*) prepared this ester in 35% yield by interaction of 9-chlorofluorene and the potassio-compound of ethyl 2-keto-*cyclo*hexanecarboxylate. We found that the yield could be increased to 75% by using 9-bromofluorene and the sodio-derivative of the keto-ester. 9-Bromofluorene was obtained in yields of 75—80% by the action of hydrobromic acid on fluorenol (Bachmann, *J. Amer. Chem. Soc.*, 1933, **55**, 2135), formed in 90% yield by hydrogenation of a solution of fluorenone in ethanol over Raney nickel. 9-Bromofluorene was obtained in 65% yield by direct bromination of fluorene with *N*-bromosuccinimide (Wittig and Felletschin, *Annalen*, 1944, **555**, 133).

Sodium (2·2 g.) was atomised in boiling purified toluene, and the toluene replaced by pure benzene (140 c.c.). Ethyl 2-keto*cyclo*hexanecarboxylate (15·9 g.) was added and the mixture kept overnight, the sodium being replaced by a voluminous precipitate of the sodio-compound. 9-Bromofluorene (23·7 g.) was then added, and the whole boiled under reflux (oil-bath) for 24 hours. The solution was slowly poured into water, and the benzene layer collected, washed, and dried ($Na_2SO_4$). The benzene was removed under reduced pressure and the residue was triturated with light petroleum (b. p. 60—80°). The undissolved solid was recrystallised from acetic acid and then had m. p. 133—134° (Hurd and Mold give m. p. 133—133·5°) (23·8 g.).

2-*Fluorenyl*cyclo*hexanone*.—Decarbethoxylation with aqueous-alcoholic potassium hydroxide gave yields of less than 20%. By use of barium hydroxide the yield of ketone was increased to 50%. A solution of ethyl 1-fluorenyl-2-keto*cyclo*hexane-1-carboxylate (10 g.) in 90% ethanol (100 c.c.) was heated under reflux with barium hydroxide (15·4 g.) for 22 hours. The solvent was distilled under reduced pressure and the residue treated with hydrochloric acid. The white solid so formed was replaced by a pale yellow oil after brief heating. This was extracted with ether. The ketone recovered from the washed and dried extract crystallised from ethanol in lustrous leaflets (3·91 g.), m. p. 111·5—112·5° (Hurd and Mold give 112·5—113·5°) (Found: C, 87·0; H, 6·9. Calc. for $C_{19}H_{18}O$: C, 87·0; H, 6·9%). The semicarbazone had m. p. 235—238° (decomp.) (Hurd and Mold give 220°) (Found: C, 75·4; H, 6·7; N, 13·5. Calc. for $C_{20}H_{21}ON_3$: C, 75·2; H, 6·6; N, 13·2%), and was hydrolysed by 6N-hydrochloric acid to the ketone of unchanged m. p.

* See footnote, p. 3168.

2-*Fluorenyl*cyclo*hexanol* (I).—(*a*) A solution of 2-fluorenyl*cyclo*hexanone (2·62 g.) in purified dioxan (40 c.c.) and copper chromite catalyst (1 g.; *Org. Synth.*, Coll. Vol. II, p. 142) was placed in a stainless steel bomb which was charged with hydrogen to 90 atm. and then heated at 150° for 1¾ hours. The filtered solution, combined with two similar batches, was evaporated and the residue was dissolved in hot *cyclo*hexane. The cooled solution gave a white solid (3·3 g.), m. p. 120—123°. After 8 recrystallisations from *cyclo*hexane this gave pure (? trans-)2-*fluorenyl*-cyclo*hexanol* as a microcrystalline powder, m. p. 126·5—127·5° (0·3 g.) (Found : C, 86·4; H, 7·6. $C_{19}H_{20}O$ requires C, 86·3; H, 7·6%). The *phenylurethane* was prepared by heating a mixture of this alcohol (0·1 g.) and phenyl *iso*cyanate at 100° for 5 minutes. After several recrystallisations from light petroleum it formed silky needles, m. p. 171·5—172·5° (Found : C, 81·6; H, 6·4; N, 3·6. $C_{26}H_{25}O_2N$ requires C, 81·4; H, 6·6; N, 3·65%). Hurd and Mold (*loc. cit.*) obtained, by reduction of the ketone with sodium and moist ether, an impure product which gave a phenylurethane, m. p. 167·5—168·5°.

Evaporation of the *cyclo*hexane liquors from the first crystallisation of the alcohol gave a yellow syrup (3·9 g.) which was dissolved in hot 95% ethanol. The cooled solution slowly deposited a white solid, m. p. 146—164°, which, after crystallisation from acetone, chloroform, and then hexane, gave *hexahydro-2-fluorenyl*cyclo*hexanol* as fine needles, m. p. 177—181° (Found : C, 84·4; H, 10·1. $C_{19}H_{26}O$ requires C, 84·4; H, 9·7%).

Concentration of the ethanol liquors from which this last material had separated gave a yellowish syrup which slowly deposited a white solid. Six recrystallisations from ethanol gave clusters of colourless needles, m. p. 122·5—123°, consisting of (? *cis*-)2-fluorenyl*cyclo*hexanol (Found : C, 86·3; H, 7·6%). The m. p. was strongly depressed by admixture with the (?)*trans*-isomer. The phenylurethane formed silky needles (from light petroleum), m. p. 145—146°, in agreement with the figure (144·5—145·5°) given by Hurd and Mold for the phenylurethane of their alcohol, m. p. 122—122·5°.

(*b*) A much more reactive copper chromite catalyst was prepared by the procedure described by Adkins, Burgoyne, and Schneider (*J. Amer. Chem. Soc.*, 1950, **72**, 2628) for their catalyst HJS2. By its means 2-fluorenyl*cyclo*hexanone could be hydrogenated at a lower temperature, yielding a much more homogeneous product. A solution of the ketone (5·24 g.) in purified dioxan (80 c.c.) was placed in a steel bomb with 2 g. of the active catalyst and charged with hydrogen at 88 atm. Hydrogenation was effected at 80° in 2 hours. The filtered solution gave on evaporation a solid which, after two recrystallisations from ethanol, formed narrow slabs (3·5 g.), m. p. 122—123°, not depressed by the (?)*cis*-alcohol (I). When hydrogenation over this active catalyst was carried out at 150° for 1¾ hours there was formed a mixture from which the (?)*trans*-alcohol and the hexahydride, m. p. 177—181°, were both isolated.

9-cyclo*Hex*-1′-*enylfluorene* (IV).—(*a*) The (?)*cis*-alcohol (I) was heated with phosphoric acid at 230° for ½ hour, as described by Hurd and Mold. The resulting 9-*cyclo*hex-1′-enylfluorene, obtained in 45% yield, crystallised from methanol in colourless needles, m. p. 110·5—111° (Found : C, 92·6; H, 7·4. Calc. for $C_{19}H_{18}$ : C, 92·7; H, 7·4%). Light absorption in ethanol : $\lambda_{max.}$ 2665, 2920, 3025 Å; log $\varepsilon$ = 4·23, 3·72, 3·85. Both micro-hydrogenation with palladium black in acetic acid and titration with monoperphthalic acid gave values corresponding with one double bond. A solution of the hydrocarbon in carbon tetrachloride reacted only slowly with bromine in the same solvent, and with aqueous permanganate in acid or neutral solution. This relative inertness of the double bond is possibly to be ascribed to steric hindrance.

(*b*) The same hydrocarbon (IV) was obtained by similar treatment with phosphoric acid of the (?)*trans*-alcohol.

(*c*) Powdered (?)*cis*-2-fluorenyl*cyclo*hexanol (1 g.) was mixed with powdered anhydrous potassium hydrogen sulphate (0·1 g.) and heated gradually to 180° in an evacuated sublimation apparatus (*ca.* 15 mm.). The sublimate was twice recrystallised from methanol and then had m. p. 110—111°, alone or mixed with the hydrocarbon prepared by use of phosphoric acid.

*Reactions of* 9-cyclo*Hex*-1′-*enylfluorene*.—(*a*) A solution of chromium trioxide (1·5 g.) in water (1·5 c.c.) and acetic acid (1 c.c.) was added dropwise to an almost boiling solution of the unsaturated hydrocarbon (IV) (0·25 g.) in acetic acid (6·5 c.c.). The solution was boiled for 1½ hours, kept overnight, and then diluted with water and extracted with ether. The product recovered from the washed and dried ethereal extract crystallised from benzene–light petroleum as yellow needles, m. p. 80—81·5°, not depressed on admixture with authentic fluorenone. Identification was completed by preparation of the oxime, m. p. 192—193·5° (lit., 195°).

(*b*) 9-*cyclo*Hex-1′-enylfluorene (0·35 g.) was dissolved in ether (60 c.c.) containing monoperphthalic acid (*ca.* 0·6 g.) (Stahmann and Bergmann, *J. Org. Chem.*, 1946, **11**, 589), and the cold solution was kept for 2 days. It was then shaken with dilute alkali, washed with

water, and dried ($Na_2SO_4$), and the ether removed. The residual solid (0·36 g.) had m. p. 86—89°. After several recrystallisations from light petroleum (b. p. 40—60°) 9-cyclo*hex*-1′-*enylfluorene epoxide* formed colourless prisms, (micro-)m. p. 89—90° (Found: C, 86·7; H, 6·9. $C_{19}H_{18}O$ requires C, 87·0; H, 6·9%).

(*c*) Osmium tetroxide (0·34 g.) was added to a solution of the unsaturated hydrocarbon (0·33 g.) in pyridine (0·21 g.) and dry benzene (3·5 c.c.). The dark brown solution soon deposited a brown osmium complex. This was collected and hydrolysed by shaking its chloroform solution with mannitol (5 g.) and potassium hydroxide (0·5 g.) in water (50 c.c.) for 2½ hours. The white solid (0·3 g.) recovered from the chloroform solution was recrystallised from methanol. 9-(1 : 2-*Dihydroxy*cyclo*hexyl*)*fluorene* formed small colourless crystals, m. p. 183—184° (Found: C, 81·5; H, 7·2. $C_{19}H_{20}O_2$ requires C, 81·4; H, 7·2%).

1-*Fluorenyl*cyclo*hexanol* (VII).—Fluorenylmagnesium bromide was prepared from fluorene (25 g.) and ethylmagnesium bromide (from 20 g. of ethyl bromide and 4·3 g. of magnesium) in xylene (Miller and Bachman, *J. Amer. Chem. Soc.*, 1935, **57**, 766). The xylene was decanted from the deposit of fluorenylmagnesium bromide, which was washed with more xylene, then covered with xylene and heated to 80°. *cyclo*Hexanone (9 g.), mixed with a little dry benzene, was added to the stirred solution. Stirring at 80° was continued for 2 hours. After being kept overnight the product was poured into cold dilute sulphuric acid. The xylene–benzene solution was worked up in the standard way, and 1-*fluorenyl*cyclo*hexanol* (6 g.) was obtained as clusters of flat colourless needles (from light petroleum, b. p. 60—80°), m. p. 130—131° (Found: C, 86·5; H, 7·7. $C_{19}H_{20}O$ requires C, 86·3; H, 7·6%). Fruitless attempts were made to prepare an acetate, a 3 : 5-dinitrobenzoate, and a phenylurethane. Dehydration with potassium hydrogen sulphate, as described for the isomer (I), gave 9-*cyclo*hex-1′-enylfluorene (IV), m. p. 110—111°, alone or mixed with a sample prepared from (I).

9-cyclo*Hexylfluorenol* (VIII).—A solution of fluorenone (2·2 g.) in dry ether was added slowly to a Grignard solution prepared from *cyclo*hexyl bromide (4·4 g.) and magnesium turnings (0·7 g.) in dry ether (50 c.c.). The solution was boiled for 3 hours and the magnesium complex then decomposed with cold ammonium chloride solution. The resulting 9-cyclo*hexylfluorenol*, isolated in the usual manner, formed colourless crystals (from aqueous methanol), m. p. 126·5—128° (Found: C, 86·5; H, 7·6. $C_{19}H_{20}O$ requires C, 86·3; H, 7·6%). The *phenylurethane* crystallised from light petroleum (b. p. 80—100°) in fine silky needles, m. p. 184—186° (decomp.) (Found: C, 81·4; H, 6·7; N, 3·8. $C_{26}H_{25}O_2N$ requires C, 81·4; H, 6·6; N, 3·7%).

9-cyclo*Hexylidenefluorene* (VI).—9-*cyclo*Hexylfluorenol (0·33 g.) was dehydrated by heating it under reduced pressure with potassium hydrogen sulphate (0·034 g.) as in the case of the isomeric alcohol (I). The resulting sublimate of 9-cyclo*hexylidenefluorene* (0·27 g.) crystallised from methanol in needles, m. p. 107—108°, depressed by admixture with the hydrocarbon formed by dehydration of 2-fluorenyl*cyclo*hexanol (Found: C, 92·6; H, 7·3. $C_{19}H_{18}$ requires C, 92·7; H, 7·3%). Light absorption in ethanol: $\lambda_{max.}$ 2330, 2500, 2595, 2870, 3185 Å; log ε = 4·67, 4·35, 4·37, 4·24, 4·14. This hydrocarbon was very unstable. The colourless crystals when kept in air slowly became yellow and finally resinous. The m. p. fell to about 90° in 24 hours.

9-cyclo*Hexylfluorene*.—(*a*) A solution of *cyclo*hexylidenefluorene (0·04 g.) in acetic acid was shaken with hydrogen and palladium black (0·05 g.). Hydrogen absorption was complete after 2 hours. The resulting 9-*cyclo*hexylfluorene had m. p. 115—115·5° (from methanol), not depressed by a specimen, m. p. 114·5—115·5°, prepared from *cyclo*hexylmagnesium bromide and 9-bromofluorene. Miller and Bachman (*loc. cit.*), who prepared 9-*cyclo*hexylfluorene by this Grignard method with 9-chlorofluorene, gave m. p. 102—103°, but this was subsequently corrected by Brown and Bluestein (*J. Amer. Chem. Soc.*, 1943, **65**, 1083) to 115—116°. Light absorption in ethanol: $\lambda_{max.}$ 2270, 2665, 2918, 3028 Å; log ε = 3·79, 4·24, 3·74, 3·88.

(*b*) 9-*cyclo*Hex-1′-enylfluorene (0·5 g.), prepared by dehydration of 2-fluorenyl*cyclo*hexanol with phosphoric acid, was likewise hydrogenated in acetic acid solution over palladium black (0·25 g.). Absorption of hydrogen was slow, and was complete only after 12 hours. The resulting 9-*cyclo*hexylfluorene had m. p. 115—115·5° (from methanol), depressed by admixture with the initial hydrocarbon, but not by admixture with authentic 9-*cyclo*hexylfluorene.

We are grateful to the Department of Scientific and Industrial Research for a Maintenance Allowance (to L. H.). Microanalyses were carried out by Mr. J. M. L. Cameron and Miss R. H. Kennaway.

UNIVERSITY OF GLASGOW. [*Received, April 24th*, 1952.]

## 606. 3-*Indolylacetaldehyde and* 3-*Indolylacetone.*

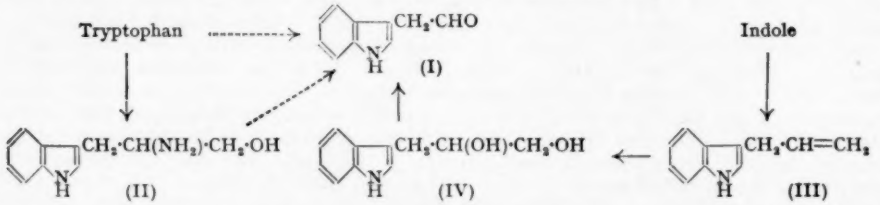
By J. B. Brown, H. B. Henbest, and E. R. H. Jones.

**3-Indolylacetaldehyde (I), the aldehyde corresponding with heteroauxin, which has been claimed to have been detected in some plant sources, has been prepared for the first time, and has been fully characterized. The related 3-indolylacetone (VI) has also been prepared.**

The tissues of several plant species have been reported to contain a neutral compound, believed by many workers to be 3-indolylacetaldehyde (I), the aldehyde related to heteroauxin; however, recent work by Bentley, Henbest, Jones, and Smith (*Nature*, 1952, **169**, 485) has shown that the neutral hormone present in cabbage and brussels sprouts is 3-indolylacetonitrile. The chief evidence for the presence of 3-indolylacetaldehyde in plants (for a comprehensive review see Larsen, *Ann. Rev. Plant Physiol.*, 1951, **2**, 176) has been the conversion of part of a neutral ether-soluble fraction into a growth-promoting acidic material (presumed to be heteroauxin) by enzymic action, although Gordon and Nieva (*Arch. Biochem.*, 1949, **20**, 356, 367) have reported that the neutral substance can be extracted by aqueous solutions of dimedone and sodium hydrogen sulphite, regeneration being possible in the latter case to yield a material again convertible into a physiologically active acid. The isolation of the aldehyde itself (or any derivatives thereof) has not been described, but Larsen has prepared a material containing about 2% of this substance (estimated by bioassay of the auxin produced by soil treatment) by treating tryptophan with ninhydrin (or isatin) in aqueous solution.

3-Indolylacetaldehyde is also of interest because it has been postulated as a possible intermediate in the conversion *in vivo* of tryptophan into heteroauxin.

In the first place the ninhydrin oxidation of tryptophan was re-investigated, but it soon became clear that a very heterogeneous product was invariably formed. In view of the probable instability of the aldehyde, attention was then directed towards milder synthetical methods, in particular, to the possibility of oxidising tryptophanol (II) (Karrer and Portmann, *Helv. Chim. Acta*, 1949, **32**, 1034) by means of periodic acid—this reagent has been employed to split *vic.*-amino-alcohols (Jackson, "Organic Reactions," Vol. 2, p. 341). Periodate oxidation of tryptophanol was conducted in water at pH ~6 in the presence of ether to remove the sensitive aldehyde as formed. However, the reaction proceeded only slowly at 20° producing much insoluble material, and the heterogeneous ether-soluble portion yielded a complex mixture of 2 : 4-dinitrophenylhydrazones.

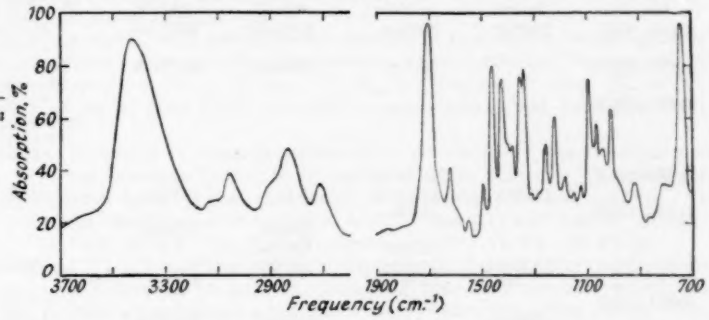



Periodate fission of the *vic.*-glycol (IV) should take place more rapidly than that of tryptophanol. This glycol was prepared from indole, by first treating the Grignard derivative of the latter with allyl bromide, producing 3-allylindole (III) in good yield. Analogy with other reactions of indolyl Grignard reagents would indicate that the allyl group is attached at the 3-position, and this was confirmed by infra-red measurements (see Experimental). Treatment of 3-allylindole with osmium tetroxide below 0° readily led to the crystalline glycol (IV). An attempt to prepared the glycol from (III) and performic acid was unsuccessful, intractable tars being obtained.

Oxidation of the glycol with periodate was conducted in the presence of ether–light petroleum in which only 3% of the glycol dissolved. The reaction proceeded rapidly at 20°, and 3-indolylacetaldehyde could be isolated readily from the organic phase. Distilla-

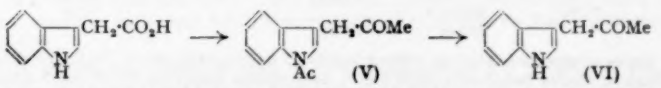
tion (attended by some material loss) gave the pure aldehyde, which was quite stable when stored at −60°. A determination of its molecular weight in benzene solution showed that it was monomeric—it was necessary to check this as phenylacetaldehyde can exist as a fairly stable trimer. The infra-red absorption spectrum is shown in the accompanying Figure. The aldehyde was further characterized as the semicarbazone, 2 : 4-dinitrophenylhydrazone, and the dimedone derivative.

From previously reported work with crude 3-indolylacetaldehyde, it is uncertain whether this compound functions as a plant-growth hormone. We are indebted to Dr. J. Bentley and Mr. S. Housley, of the Botany Department of this University, for the following preliminary report of the activity of the pure compound in the *Avena* straight-growth test (Bentley, *J. Exptl. Bot.*, 1950, **1**, 201). 3-Indolylacetaldehyde itself appears to be either inactive or inhibitory in this test. Solutions of the aldehyde cause some growth of *Avena* coleoptile sections, but an acidic substance has been shown to be produced during the test, which, assuming that this is heteroauxin, would account for all (and sometimes even more of) the activity shown by the aldehyde test solution. The amount of acid produced was determined by extracting the final test solution with ether, followed by separation into an acidic and a neutral fraction in the usual way, and evaluation of the acid by the straight-growth test. It was noteworthy that the amount of acid formed was greater in the presence of coleoptile sections than in their absence.




3-*Indolylacetaldehyde—infra-red absorption spectrum.*

It was of chemical and possible biological interest to prepare the related compound, 3-indolylacetone (VI). It was hoped that hydration of the triple bond of 3-propargylindole would give this ketone, but an attempt to prepare the propargyl compound by treating indolylmagnesium bromide with propargyl bromide failed, chiefly because the Grignard reagent reacted first with the active acetylenic hydrogen atom to regenerate indole.




Phenylacetone has been prepared by heating phenylacetic acid with acetic anhydride in the presence of sodium acetate (this reaction has been recently reviewed by King and McMillan, *J. Amer. Chem. Soc.*, 1951, **73**, 4911). Application of the method to 3-indolylacetic acid gave a 45% yield of a ketonic product, analysis and infra-red examination of which showed that it was *N*-acetyl-3-indolylacetone (V). This compound could be hydrolysed readily to (crystalline) 3-indolylacetone (VI), which was virtually inactive in the *Avena* straight-growth test.

## Experimental

M. p.s were taken on a Kofler block and are corrected. Ultra-violet light-absorption spectra were determined with a Unicam SP 500 spectrophotometer, ethanolic solutions being used, and infra-red spectral measurements were made with a Perkin-Elmer double-beam instrument (sodium chloride prism), either a liquid or a supercooled melt being used.

**3-*Allylindole*** (III).—Ethylmagnesium bromide (representing a 20% excess over the indole

to be used) was prepared from magnesium (6·5 g.) in ether (80 c.c.) and ethyl bromide (30 g.) in ether (30 c.c.). Redistilled indole (25·8 g.) in benzene (50 c.c.) was then added slowly with stirring. The indolylmagnesium bromide solution was stirred for a further 20 minutes, and redistilled allyl bromide (29·2 g.) in benzene (20 c.c.) was then added slowly, a mild exothermic reaction taking place. After the mixture had been stirred at 20° overnight, the product was isolated with ether. Distillation gave 3-allylindole (22·7 g., 70%) (together with a little unchanged indole), b. p. 94—98°/0·03 mm., $n_D^{19}$ 1·5998. Completely satisfactory analytical data could not be obtained with this compound, carbon values being somewhat high and hydrogen values low. Light absorption : Maxima, 2200, 2740, and 2860 Å; $\varepsilon$ = 35,000, 6400, and 5300, respectively.

For hydrogenation, a solution of 3-allylindole (1·71 g.) in ethanol (50 c.c.) was shaken with Adams's catalyst (0·1 g.) in an atmosphere of hydrogen. Hydrogenation was rapid and ceased after 273 c.c. had been absorbed (theoretical for 1 mole, 276 c.c.). Filtration and evaporation of the solution gave 3-propylindole (1·72 g.). Light absorption : Maxima, 2230, 2820, and 2900 Å; $\varepsilon$ = 33,800, 5900, and 5000, respectively.

*Infra-red absorption (main bands only).*

(s = strong; m = medium; w = weak, are given as approximate indications of intensity.)

| Spectrum region, cm.$^{-1}$ | Indole | 2-Methylindole | 3-Methylindole | 3-*n*-Propylindole | 3-Allylindole | 3-Indolylacetaldehyde |
|---|---|---|---|---|---|---|
| 3100—3000 | 3050m | 3060m | 3065mw | 3055mw | 3080ms, 2900ms | 3065mw |
| 3000—2900 | | 2950m | 2940m | 2970ms | (vinyl, CH stretching) | 2830m |
| 2900—1600 | | | | | 1625s (aliphatic C : C stretching) | 1705s (C = O stretching) |
| 1600—1400 | | 1550ms * | | | 1620ms | |
| | 1420m | 1410s * | | 1425m | 1425s | 1425s |
| 1400—1200 | | 1390m | | | | |
| | | | 1345m | 1335m | | 1355s |
| | | 1290ms | 1300m | | | 1340s |
| | 1250ms | 1240m | 1250m | | 1260ms | 1255m |
| | | 1220ms | | 1225m | | 1225ms |
| 1200—1000 | | 1150m | | | 1130m | |
| | 1090ms | | 1090s * | 1090ms * | 1090s | 1095s |
| | | | | | | 1070ms |
| | | 1035m | | | 1050ms | 1040m |
| 1000—700 | | | | | 995s | |
| | | | 975m | | | |
| | 935m | 925m | 925m | | 914s | |
| | | | | | (vinyl, CH bending) | |
| | | | 802m | | 810ms | |
| | 770s | 780vs * | | | 770s | |
| | | 750vs | 755m | | | |

All the above compounds showed strong bands at 3400—3450 cm.$^{-1}$ (NH stretching) and ~1460 cm.$^{-1}$, medium to strong bands at ~1350 cm.$^{-1}$, very strong bands at ~740 cm.$^{-1}$ (resolved into two bands at 730 and 750 with indole), and all except indole showed medium to strong bands at ~1010 cm.$^{-1}$

* Strong intensity bands differentiating 2- and 3-alkylindoles.

**3-3′-*Indolylpropan*-1 : 2-*diol*** (IV).—A solution of 3-allylindole (1·3 g.) and pyridine (2 c.c.) in ether (20 c.c.) was cooled to *ca.* −30° and added to a solution of osmium tetroxide (2 g.) in ether (200 c.c.) also at −30°. The solution was then allowed to warm to 15° during 1 hour, during which time the addition complex (4·3 g.) separated as a light brown solid. This was dissolved in chloroform (35 c.c.) and shaken with mannitol (30 g.) in water (300 c.c.) containing potassium hydroxide (3 g.) for 10 minutes. The chloroform layer was rejected and the aqueous phase was extracted continuously with ether for 6 hours. Evaporation of this extract yielded a gum which readily solidified. This was triturated twice with ether–light petroleum (b. p. 40—60°) (1 : 1; 10 c.c. portions) to give a slightly coloured granular solid (1·35 g.), m. p. 93—97°. Recrystallization of this from benzene gave the pure *glycol* as plates, m. p. 97·5—98° (Found : C, 69·0; H, 7·1. $C_{11}H_{13}O_2N$ requires C, 69·1; H, 6·85%). Light absorption : Maxima, 2240, 2820, and 2900 Å; $\varepsilon$ = 33,800, 5900, and 5000, respectively. When a solution of the glycol

(50 mg.) in water (30 c.c.) was shaken with an equal volume of ether, 40% of the glycol went into the ethereal layer; when a 1 : 1 mixture of ether–light petroleum (b. p. 40—60°) was used only 5% was extracted (cf. following experiment).

3-*Indolylacetaldehyde* (I) *and Derivatives.*—Before the reaction was begun, a high-vacuum line was evacuated to $10^{-5}$ mm. and an air-bath warmed to 120°. Aqueous sodium metaperiodate solution (0·1N; 26 c.c.) was stirred with ether–light petroleum (b. p. 40—60°) (1 : 1; 30 c.c.) in an atmosphere of nitrogen in a flask with a side-arm fused in at the level of the interface between the two layers of reaction solution. A solution of the foregoing glycol (0·5 g.) in water (30 c.c.) was added, the temperature of the stirred mixture rising from 14° to 18°. Stirring was continued for 5 minutes, and the organic layer was then removed *via* the side-arm, two portions of ether (30 c.c.) being added to the stirred aqueous phase to remove the remainder of the product. The combined extract was dried ($Na_2SO_4$) and evaporated under reduced pressure to leave substantially pure aldehyde (0·29 g.) as a colourless syrup. The pure compound was obtained by distillation [at 120° (bath temp.)] from a short-path still, but much polymerization appeared to take place during this operation, even when it was conducted as rapidly as possible, for the yield of distillate was only 0·09 g. (from 0·29 g.). Pure 3-*indolylacetaldehyde* had $n_D^{18}$ 1·6178 (Found: C, 75·15; H, 5·3. $C_{10}H_9ON$ requires C, 75·4; H, 5·7%). Light absorption: Maxima, 2220, 2800, and 2890 Å; $\varepsilon$ = 32,600, 6000, and 5000, respectively.

The 2 : 4-*dinitrophenylhydrazone* formed readily, but crystallized only slowly from supersaturated solutions in aqueous dioxan as yellow-brown plates, m. p. 196—202° (Found: C, 56·75; H, 4·0. $C_{16}H_{13}O_4N_5$ requires C, 56·6; H, 3·85%). Light absorption: Maxima, 2680 and 3580 Å; $\varepsilon$ = 16,800 and 23,600, respectively.

The *semicarbazone* was prepared with semicarbazide acetate in aqueous methanol solution. Recrystallization from aqueous methanol gave long needles, m. p. 142—155° unchanged on further recrystallization (Found: C, 61·1; H, 6·0. $C_{11}H_{12}ON_4$ requires C, 61·1; H, 5·6%). Light absorption: Maxima, 2210, 2730, 2800, and 2900 Å; $\varepsilon$ = 42,900, 6900, 6950, and 5650, respectively.

A saturated solution of dimedone in aqueous methanol (1 : 1) containing the aldehyde was set aside at 20° overnight; the *dimedone* derivative separated (dilution with water increased the yield) and after crystallization from aqueous methanol or chloroform formed needles. To obtain consistent m. p. values, the material was dried at 120°/20 mm.; it then had m. p. 148—152° (Found: C, 73·7; H, 7·45; N, 3·2. $C_{26}H_{31}O_4N$ requires C, 74·1; H, 7·4; N, 3·3%).

*N-Acetyl-3-indolylacetone* (V).—A mixture of 3-indolylacetic acid (2 g.), freshly fused sodium acetate (1·55 g.), and acetic anhydride (5 c.c.) was heated at 135—140° (oil-bath temp.) for 18 hours. The cooled mixture was treated with water and chloroform–ether (1 : 4). The organic layer was washed with saturated aqueous potassium hydrogen carbonate (3 × 20 c.c.), and then dried ($Na_2SO_4$) and evaporated under reduced pressure. Distillation from a short-path still gave the *ketone* (1·1 g.) as a pale yellow viscous syrup, b. p. 120° (bath temp.)/$10^{-5}$ mm., $n_D^{19}$ 1·5953 (Found: C, 72·05; H, 6·25. $C_{13}H_{13}O_2N$ requires C, 72·55; H, 6·1%). Light absorption: Maxima, 2380, 2910, and 3000 Å; $\varepsilon$ = 16,800, 6500, and 6700, respectively (cf. light absorption of *N*-acetyl-2 : 3-dimethylindole: Maxima, 2450, 2940, and 2990 Å; $\varepsilon$ = 17,300, 5200, and 5100, respectively). This distilled product, which may have contained a little of the enol-acetate, was used for the next stage (hydrolysis to 3-indolylacetone) without further purification.

The *N*-acetyl ketone was characterized by the formation of a 2 : 4-*dinitrophenylhydrazone*, which crystallized from ethyl acetate as orange-yellow needles, m. p. 194—195° (Found: C, 57·75; H, 4·6. $C_{19}H_{17}O_6N_5$ requires C, 57·7; H, 4·35%). The *semicarbazone* crystallized slowly from "Methyl Cellosolve" as a granular solid, m. p. 208—211° (Found: C, 61·75; H, 6·0. $C_{14}H_{16}O_2N_4$ requires C, 61·75; H, 5·9%).

3-*Indolylacetone* (VI).—N-Methanolic sodium methoxide (0·8 c.c.) was added to a solution of *N*-acetyl-3-indolylacetone (0·93 g.) in dry methanol (60 c.c.). After being kept at 40° for 10 minutes, the solution was acidified with dilute hydrochloric acid and extracted with ether. The ethereal extract was processed in the usual way to yield a solid product which after one recrystallization from aqueous methanol (1 : 1) weighed 0·41 g. and had m. p. 113—115°. Further recrystallisation from the same solvent gave the *ketone* as needles, m. p. 115—117·5° (Found: C, 76·0; H, 6·45. $C_{11}H_{11}ON$ requires C, 76·3; H, 6·4%). Light absorption: Maxima, 2210, 2800, and 2890 Å; $\varepsilon$ = 35,100, 6400, and 5300, respectively. Infra-red spectrum: 3380 cm.$^{-1}$ (strong) (NH stretching); 1706 cm.$^{-1}$ (strong) (C:O stretching). The 2 : 4-*dinitrophenylhydrazone* crystallized from methanol–ethyl acetate as orange-red prisms, m. p. 161—163° (Found: C, 58·1; H, 4·2. $C_{17}H_{15}O_4N_5$ requires C, 57·8; H, 4·3%).

The biological testing of the compounds described in this paper was made possible by a grant from the Agricultural Research Council. The tests were made in the Botany Department of this University, under the direction of Professor S. C. Harland, F.R.S., by Dr. J. A. Bentley and Mr. S. Housley.

The infra-red spectra were determined by Dr. G. D. Meakins, and the microanalyses by Mr. E. S. Morton and Mr. H. Swift One of the authors (J. B. B.) thanks the Department of Scientific and Industrial Research for a Maintenance Grant.

THE UNIVERSITY, MANCHESTER, 13. [*Received, April 23rd,* 1952.]

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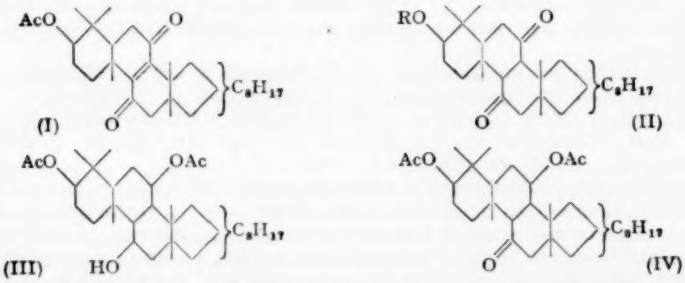
## 607. *Lanosterol. Part XIV.* Further Experiments on the Reduction of Ketones in the Lanosterol Series.*

By J. F. McGHIE, M. K. PRADHAN, and (in part) J. F. CAVALLA.

Reduction of acetoxylanostanedione by the modified Wolff–Kishner method (Herr, Whitmore, and Schliessler, *J. Amer. Chem. Soc.*, 1945, **67**, 2061) gave acetoxylanostanone, which on further reduction yielded acetoxylanostanol. This, on dehydration with phosphorus oxychloride followed by hydrogenation, gave acetoxylanostane (Ruzicka *et al.*, *Helv. Chim. Acta*, 1950, **33**, 1893). Similar reduction of acetoxylanostenedione gave acetoxylanostenone, which is isomeric with the " ketolanostenyl acetate " obtained by chromic acid oxidation of lanostenyl or γ-lanostadienyl acetate (Marker, Wittle, and Mixon, *J. Amer. Chem. Soc.*, 1937, **59**, 1368; Cavalla and McGhie, *J.*, 1951, 744). Lanostadienone on reduction by the modified Wolff–Kishner method, gave lanostadiene.

THE investigations now described are a continuation of others (*J.*, 1951, 744, 834, 3142), in which reduction of certain ketones derived from lanostadienol was reported.

It is known that acetoxylanostenedione (I) is easily reduced under mild conditions to the saturated lanostanedionol (II; R = H) (Dorée McGhie, and Kurzer, *J.*, 1948, 988). Further reduction of (II; R = Ac) with lithium aluminium hydride in anhydrous ether, followed by acetylation, gave diacetoxylanostanol (III) (Cavalla and McGhie, *J.*, 1951, 834; Voser, Montavon, Günthard, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 1893), whereas reduction of acetoxylanostenedione (I) under similar conditions yielded diacetoxylanostanone (IV) (Cavalla and McGhie, *loc. cit.*). The $C_{(8)}$- and the $C_{(11)}$-keto-group therefore differ in reactivity. This has been confirmed by preparation of a monoxime by Voser *et al.* (*loc. cit.*).

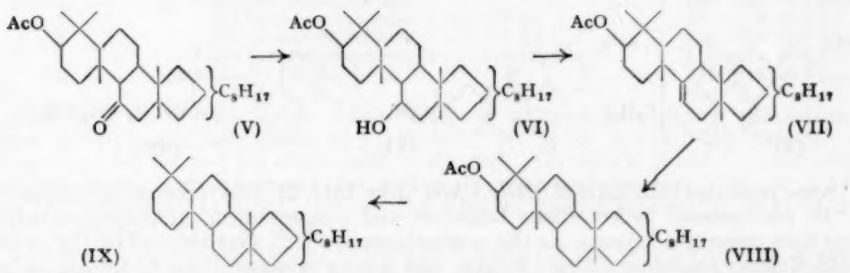



Whilst our experiments on the conversion of acetoxylanostanedione into acetoxylanostanone and thence into lanostanol were in progress, the Swiss workers reported such a conversion into acetoxylanostanone (V) (Voser *et al.*, *loc. cit.*). The melting point of (V) reported varied from 140° to 151°, apparently depending on the method of isolation.

* Part XIII, *J.*, 1951, 3142.

They reduced acetoxylanostanone with lithium aluminium hydride to the corresponding diol, the acetyl derivative (VI) of which was dehydrated with phosphorus oxychloride to acetoxylanostene (VII), whence catalytic reduction gave acetoxylanostane (VIII); from this the fundamental hydrocarbon lanostane (IX) was prepared.

In a previous communication (Cavalla, McGhie, and Pradhan, *loc. cit.*) it was shown that ketolanostenyl acetate (X) is easily reduced by the modified Wolff–Kishner method (Herr, Whitmore, and Schliessler, *loc. cit.*) to *iso*lanostenyl acetate. As the keto-group at $C_{(8)}$ is less hindered than that at $C_{(11)}$ (for a discussion on this point see Barton, Fawcett, and Thomas, *loc. cit.*) and is easily reducible by the Wolff–Kishner method, we subjected acetoxylanostanedione (II; R = Ac) to the modified Wolff–Kishner procedure: Acetoxylanostanedione hydrazone (which was not isolated) was heated with sodium in diethylene glycol at 230°. The product, on acetylation followed by chromatographic purification, gave colourless needles, $C_{32}H_{54}O_3$, m. p. 150—152°, $[\alpha]_D$ +62·9°, identical with acetoxylanostanone (V). Confirmation was obtained by hydrolysis to the corresponding lanostanonol, m. p. 161—162°, $[\alpha]_D$ +60·0°. Reduction of either acetoxylanostanone or lanostanonol with lithium aluminium hydride gave the diol, which, as above, gave (VI) and (VII), m. p. 177—178°, $[\alpha]_D$ +83·0°. After initial difficulties (VII) was hydrogenated to acetoxylanostane (VIII) in glacial acetic acid at 80°, without pre-reduction of the catalyst. The annexed table is a comparison of our products with those of Voser *et al.* (*loc. cit.*).

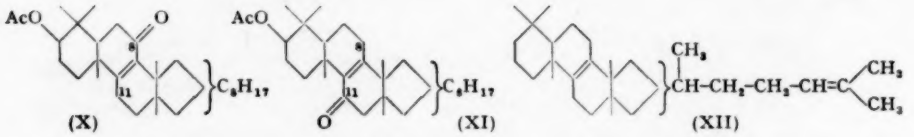



| | Voser *et al.* | | Present work | |
|---|---|---|---|---|
| | M. p. | $[\alpha]_D$ | M. p. | $[\alpha]_D$ |
| Acetoxylanostanone | 143—144° | +60·0° | 150—152° | +62·0° |
| Lanostanonol | 160—162 | +62·0 | 161—162 | +60·0 |
| Acetoxylanostanol | 219—220 | +23·0 | 215—216 | +22·8 |
| Acetoxylanostene | 170—171 | +18·0 | 177—178 | +83·0 |
| Acetoxylanostane | 150—151 | +41·0 | 156—157 | +45·0 |

We then turned our attention to the unsaturated diketone, acetoxylanostenedione, where we expected difficulty since it is well known that αβ-unsaturated carbonyl compounds usually give the pyrazolines (Lardelli and Jeger, *Helv. Chim. Acta*, 1949, **32**, 1817) rather than the hydrazones on reaction with hydrazine, the former being converted by hot alkali into *cyclo*propane derivatives. Since, however, carvone, carvenone, and *cyclo*pentenone behave normally (Merejkowsky, *Bull. Soc. chim.*, 1925, **37**, 1174) it has been suggested (Merejkowsky, *loc. cit.*) that *cyclo*propane formation does not take place if it requires the formation of one ring within another. These conditions apply to acetoxylanostenedione, and since the αβ-unsaturated ketone group of ketolanostenyl acetate (X) behaved normally in the Wolff–Kishner reduction, we attempted the reduction of acetoxylanostenedione (I) by the modified Wolff–Kishner process: we isolated a major and a minor component. The former, long white needles, m. p. 119—120°, $[\alpha]_D$ +131·3°, had the formula $C_{32}H_{52}O_3$ of a ketolanostenyl acetate. Its ultra-violet absorption spectrum indicated an αβ-unsaturated ketone by an intense band at 255 mμ (log ε 3·9) and another less intense at 302 mμ (log ε 1·6) (Campbell and Harris, *J. Amer. Chem. Soc.*, 1941, **63**, 2721; Clifford and Woodward, *ibid.*, p. 2727). The αβ-unsaturated ketonic character of the

compound was confirmed by infra-red absorption bands at 1736, 1656, 1583, and 1243 cm.$^{-1}$. The 1736- and 1243-cm.$^{-1}$ bands are due to the C=O and C—O vibrations, respectively, of the acetate group, that at 1656-cm.$^{-1}$ to the ketone in conjugation with C=C, and that at 1583 cm.$^{-1}$ to the C=C group. We are indebted to Mr. S. F. D. Orr (Chester Beatty Research Institute) for these measurements. This compound is isomeric with ketolanostenyl acetate (X), m. p. 152—153°, $[\alpha]_D$ +18·5°, which is known to have the keto-group at $C_{(8)}$ (Barton, Fawcett, and Thomas, *loc. cit.*), and arises by the reduction of the unsaturated 8 : 11-diketone (I); it must of necessity be $C_{(11)}$-ketone (XI).

The major component, m. p. 158—160°, $[\alpha]_D$ +78°, had absorption maxima at 235, 243, and 253 mμ (log ε 3·8, 3·9, and 3·8 respectively), indicating that it was impure γ-lanostadienyl acetate. This was obtained by Barton *et al.* as a by-product during the normal Wolff–Kishner reduction of ketolanostenyl acetate, and we think that in this instance it also arises from the isomeric ketolanostenyl acetate (acetoxylanostenone) (XI).




Some years ago Ruzicka *et al.* (*Helv. Chim. Acta*, 1944, **27**, 479) reported the preparation of the fundamental hydrocarbons lanostene and γ-lanostadiene (dihydroagnostadiene) from their respective ketones, *via* the semicarbazones which were reduced by the classical Wolff–Kishner procedure (Dorée, McGhie, and Kurzer prepared these hydrocarbons by a modified Clemmensen method; *J.*, 1947, 1467). We have now obtained them in excellent yields by the modified Wolff–Kishner method, in which large quantities can be reduced with comparative ease in a much shorter time. We also report the similar preparation of the hitherto undescribed hydrocarbon, lanostadiene (XII).

## Experimental

M. p.s are uncorrected. Specific rotations were determined on chloroform solutions at 20°. The aluminium oxide used in the chromatographic work was from Messrs. Peter Spence Ltd. (Grade "O"). Analyses are by Drs. Weiler and Strauss, Oxford. Light petroleum refers to the fraction of b. p. 60—80°. Ultra-violet absorption spectra were measured with a Unicam S.P. 500 spectrophotometer. Infra-red absorption spectra were determined on carbon disulphide and chloroform solutions. The term "in the usual manner" below refers to dilution with water, extraction with ether, washing successively with aqueous sodium hydroxide solution, aqueous hydrochloric acid, and water, drying of the ethereal solution ($Na_2SO_4$), and removal of the solvent *in vacuo*.

*Modified Wolff–Kishner Reduction of Acetoxylanostanedione.*—Acetoxylanostanedione (7·0 g.) in diethylene glycol (250 ml.) was heated under reflux with hydrazine hydrate (3·5 ml.; 100%) at 200° for 1 hour. The mixture was cooled, a solution of sodium (7·0 g.) in diethylene glycol (70 ml.) was added, and the mixture heated under reflux at 220—230° for 6 hours. The solution was allowed to cool, then diluted with cold water, acidified, and extracted with ether. The ethereal solution was washed well with water and dried ($Na_2SO_4$). Removal of the ether gave a light brown solid (5·0 g.). This was acetylated with pyridine (50 ml.) and acetic anhydride (50 ml.) on a steam-bath for 3 hours. The product was worked up in the usual manner, to give a dark brown oil, which was adsorbed on alumina (14 × 1·3 cm.) from light petroleum; elution with light petroleum (200 ml.) gave a colourless acetate, m. p. 142—145° (from chloroform–methanol). This, on recrystallisation from the same solvent, gave pure acetoxylanostanone as needles (3·3 g.), m. p. 150—152°, $[\alpha]_D^{20}$ +62·9° (*c*, 1·074) (Found: C, 78·9; H, 11·5. Calc. for $C_{32}H_{54}O_3$: C, 78·96; H, 11·2%).

*Acetoxylanostanone* ⟶ *Acetoxylanostane.*—Acetoxylanostanone was converted, essentially by the methods of Voser *et al.*, into lanostanol, lanostanediol, m. p. 190—191°, $[\alpha]_D^{20}$ +28·4° (*c*, 0·706), acetoxylanostene, and acetoxylanostane (see the Table, p. 3177, for constants).

*Modified Wolff–Kishner Reduction of Acetoxylanostenedione.*—Acetoxylanostenedione (4·8 g.)

in diethylene glycol (80 ml.) was heated with hydrazine hydrate (4 ml.; 60%) for 1 hour at 200°. The mixture was cooled, a solution of sodium (5·0 g.) in diethylene glycol (80 ml.) was added, and the mixture heated under reflux at 220° for 6 hours. The mixture was then cooled, diluted with water, acidified, and extracted with ether. The ethereal solution was washed well with water and dried ($Na_2SO_4$). Removal of the ether gave a brown solid. This was acetylated with pyridine (25 ml.) and acetic anhydride (75 ml.) on a steam-bath for 4 hours. The mixture was then worked up in the usual manner, to give a brown oil. This was adsorbed on alumina (18 × 1·3 cm.) from light petroleum; elution with light petroleum (300 ml.), followed by light petroleum–benzene, gave material (A) (2·1 g.) melting between 110° and 116° after crystallisation from chloroform–methanol; further elution by light petroleum–benzene (1 : 1) gave material (B) (0·05 g.), m. p. 142—147° after crystallisation from chloroform–methanol. Further chromatography of (A) gave the following results (m. p.s after crystallisation from methanol):

| Fraction | Eluant | Product, m. p. |
|---|---|---|
| 1 | Light petroleum, 100 ml. | 118—136° (0·1 g.) |
| 2 | Light petroleum, 200 ml. | 150—152° (0·1 g.) |
| 3 | Light petroleum–benzene (7 : 3), 100 ml. | 110—112° (0·2 g.) |
| 4 | Light petroleum–benzene (1 : 1), 100 ml. | 112—115° (0·8 g.) |
| 5 | Light petroleum–benzene (3 : 7), 100 ml. | 112—114° (0·6 g.) |
| 6 | Benzene, 100 ml. | 114—116° (0·1 g.) |

Fraction 2 on recrystallisation from chloroform–methanol gave impure γ-lanostadienyl acetate (0·05 g.), glistening plates, m. p. 158—160°, $[\alpha]_D^{20}$ +78° (*c*, 1·04), $\lambda_{max.}$ 235, 243, and 253 mμ (log ε 3·8, 3·9, and 3·8, respectively, in ethanol). Fractions 4, 5, and 6, on recrystallisation from methanol, gave long colourless needles of pure *acetoxylanostenone* (1·2 g.), m. p. 119—120°, $[\alpha]_D^{20}$ +131·3° (*c*, 1·08), $\lambda_{max.}$ 255 mμ (log ε 3·9, in ethanol) (Found: C, 79·2; H, 10·9. $C_{32}H_{52}O_3$ requires C, 79·3; H, 10·8%).

*Lanostene.*—Lanostenone (2·0 g.) in diethylene glycol (50 ml.) was heated with hydrazine hydrate (2 ml.; 100%) at 200° for 30 minutes. The mixture was allowed to cool, a solution of sodium (2·0 g.) in diethylene glycol (20 ml.) added, and the whole refluxed for a further 6 hours. The mixture was worked up in the usual manner, to give a yellow glass and on crystallisation from acetic acid pure lanostene (1·8 g.) as plates, m. p. 74—75°, $[\alpha]_D^{20}$ +65·0° (*c*, 1·02) (Found: C, 87·3; H, 12·5. Calc. for $C_{30}H_{52}$: C, 87·4; H, 12·6%), which gave no depression in m. p. on admixture with an authentic specimen.

*γ-Lanostadiene.*—γ-Lanostadienone (10·0 g.), reduced by the modified Wolff–Kishner method, gave γ-lanostadiene (8·0 g.), plates (from glacial acetic acid), m. p. 93—95°, $[\alpha]_D^{20}$ +75·4° (*c*, 1·02) (Found: C, 87·6; H, 12·2. Calc. for $C_{30}H_{50}$: C, 87·8; H, 12·2%), which gave no depression in m. p. on admixture with an authentic specimen.

*Lanostadiene.*—Lanostadienone (2·0 g.) in diethylene glycol (25 ml.) was treated with hydrazine hydrate (2 ml.; 100%) and sodium (2·09 g.) in diethylene glycol (25 ml.) as above. The mixture was cooled, poured into water, acidified, and extracted with ether. The ethereal solution was washed with water and dried ($Na_2SO_4$). Removal of the ether gave a sticky solid. This was adsorbed on alumina (12 × 1·3 cm.) from light petroleum; elution with light petroleum (300 ml.) gave a colourless oil; two crystallisations from chloroform–methanol gave *lanostadiene* as plates (1·8 g.), m. p. 79·5—80°, $[\alpha]_D^{20}$ +65·2° (*c*, 0·981) (Found: C, 87·7; H, 12·2. $C_{30}H_{50}$ requires C, 87·8; H, 12·2%).

One of us (J. F. C.) is indebted to the Department of Scientific and Industrial Research for a maintenance award.

CHELSEA POLYTECHNIC, MANRESA ROAD, LONDON, S.W.3. [*Received, April 10th*, 1952.]

## 608. *The Chemistry of the Wood Cell Wall. Part II.* The Isolation of Beech and Spruce Acid-soluble and Modified Lignins.†*

By (the late) W. G. CAMPBELL and I. R. C. MCDONALD.

From the sulphuric acid lignin hydrolysates of beech and spruce woods, beech and spruce acid-soluble lignins have been isolated by a new method. Similarly, beech and spruce modified lignins have been prepared from the sulphuric acid lignin hydrolysates of the corresponding "holocelluloses." Therefore the total lignin content of beech or spruce wood or of their holocelluloses cannot be estimated by the 72% sulphuric acid method. The methoxyl and the carbon contents of these substances are considerably lower than those of the sulphuric acid lignins from the same woods.

THE previous paper in this series * drew attention to the presence in partly delignified beech- and spruce-wood residues ("holocelluloses") of a form of lignin which was not isolable by the usual method of lignin determination. This material was called "modified lignin" because it was considered to be a form of lignin so modified during the delignification of wood by buffered aqueous sodium chlorite that it was no longer isolated as an insoluble residue by the normal 72% sulphuric acid method for determination of lignin. It was further shown that the true total polysaccharide content of either wood, as a percentage, was less than the value $100 - x$ (where $x$ is the percentage of acid lignin) for the probable reason that a portion of the native lignin present in these woods is not recoverable as acid lignin. This suggested that a fraction of native lignin is "acid soluble."

Various authors (Hägglund, *Papierfabrikant*, 1925, **23**, 406; *Chem. Abs.*, 1925, **19**, 3015; Bamford and Campbell, *Biochem. J.*, 1936, **30**, 419) have remarked on the difficulty of filtering the sulphuric acid lignin precipitate from wood and have observed that subsequent flocculation occurs in the filtered lignin hydrolysates unless very fine filters are used. Migita and Kawamura (*J. Agric. Chem. Soc. Japan*, 1944, **20**, 348, 507) reported that the acid lignin fraction of wood could be fractionally precipitated if the acid strength was reduced stepwise from 72 to 3%. A further precipitate was formed when the filtered 3% mixture was boiled, but these authors did not suggest that an additional lignin fraction still remained in solution. The acid lignin hydrolysates from beech- or spruce-wood are known to give ultra-violet absorption spectra regarded as characteristic of lignin and also, in the case of beech-wood, a pronounced Maüle colour reaction. It has been observed that similar hydrolysates from the lignin determinations on beech- and spruce-wood residues, which have been partly delignified by buffered sodium chlorite solution ("holocelluloses"), also have these properties. Von Wacek and Schroth (*Holz als Roh-und Werk-stoff*, 1951, **9**, No. 1, 7; *Das Papier*, 1950, **4**, 410; *Monatsh.*, 1950, **81**, 1151) have reported the presence of a soluble form of lignin (which was called protolignin I, after Jayme) in the acid lignin filtrates of spruce wood and also in alkaline extracts of *Entandrophragma utile* wood. These authors state that they could not isolate this soluble lignin from the acid lignin hydrolysates but have isolated it from the alkaline wood extracts. Although some 5·5% of it has been isolated from spruce wood, methoxyl balance was used to calculate the amount of this material in the tropical hardwood.

In experiments in this laboratory it was observed that the ultra-violet absorption spectrum of the acid lignin hydrolysates of beech wood had a definite maximum at 277·5, a minimum at 255, and inflexions at 320 and 225 mμ (Fig. 1). If this acid solution was passed through an ion-exchange resin column (Zeo-Karb 215) the resulting acid solution showed greatly reduced absorption throughout the entire spectrum. The maximum, the minimum, and the inflexion at 225 mμ are, however, still present (Fig. 1). The original solution gave a pronounced Maüle reaction (chlorine and sodium sulphite) whereas after passing through the column the solution gave a very weak pink colour with

* Part I, *J.*, 1952, 2644.

† This paper was in part presented by the late W. G. Campbell at the Lignin Session of the International Pulp and Paper Symposium, Appleton, Wisconsin, September 1951.

these reagents. Absorption curves similar to those in Fig. 1 have also been obtained for the sulphuric acid lignin hydrolysate from spruce wood before and after passage through an ion-exchange column.

From these observations a simple method has been devised by which the materials responsible for the major part of the absorption of the original sulphuric acid lignin hydrolysates of beech or spruce woods have been isolated as buff to pale brown powders or

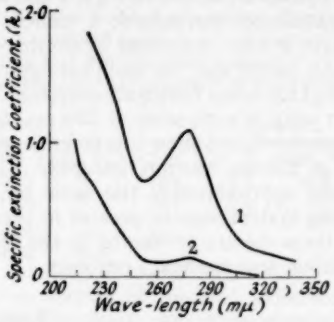
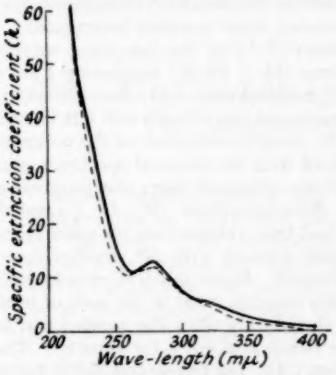



FIG. 1.

1, *Original sulphuric acid lignin hydrolysate.*
2, *Solution, as above, after passage through Zeo-Karb* 215.

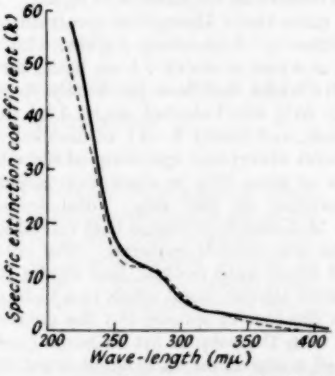
as brown to red resinous products. These materials are not polysaccharides or mixtures of sulphuric acid lignins and polysaccharides. Therefore, because these materials were also soluble in 3% sulphuric acid, under the conditions prevailing in the 72% sulphuric acid method, the names " beech and spruce acid-soluble lignin " have been assigned to them. These materials, when isolated in the powder form, are labile compounds, which are easily converted by warming into resinous products. In the case of beech this conversion is evidently a polymerisation because both the resinous and the powder form give the same

FIG. 2.




——— *Beech acid-soluble lignin.*
- - - - - *Beech modified lignin.*

FIG. 3.




——— *Spruce acid-soluble lignin.*
- - - - - *Spruce modified lignin.*

analytical figures. Beech acid-soluble lignin has been isolated from the lignin hydrolysates in yield equivalent to 7·4% by weight of the original wood and spruce acid soluble lignin in 5·8% yield calculated on a similar basis.

Similarly, beech modified lignin has been isolated from the acid lignin hydrolysates of samples which have been partly delignified with sodium chlorite. Wood residues obtained in yields of 92·9, 87·4, and 71·9% gave respectively 14·0, 7·2, and 0·7% of sulphuric acid lignin and 8·7, 10·0, and 5·0% of modified lignin.

In view of the difficulties in determining whether a lignin preparation is homogeneous, analyses should be examined with caution (see p. 3183). Tentative formulæ have

been calculated as follows, on the assumption that the materials are in fact homogeneous: beech acid-soluble lignin, $[C_{40}H_{43}O_{20}(OMe)_5]_n$; beech modified lignin, $[C_{40}H_{43}O_{20}(OMe)_5,2HCl]_n$. Similar formulæ are not possible for the spruce acid-soluble and modified lignins on the results so far obtained.

*Ultra-violet Absorption Spectra of Acid-soluble and Modified Lignins.*—The ultra-violet absorption spectra of beech acid-soluble and modified lignins and spruce acid-soluble and modified lignins are shown in Figs. 2 and 3. As the molecular weights were not known the specific extinction coefficients, $k$ (concn. calc. as g./l.), have been plotted against the wave-length in mμ. In either figure there is little difference between the spectrum of the acid-soluble lignin and the modified lignin, indicating that the addition of $Cl_2$ or HCl has not affected the main conjugating system. While in the case of the beech samples (Fig. 2) a distinct peak is noticeable at 280 mμ, in addition to inflexions at 320 and 225 mμ, the spruce samples do not show this definite peak—the inflexion at 320 mμ is, however, present, but that at 225 mμ was not detected. The specific extinction coefficients over the entire spectra are approximately the same for all the substances, suggesting that the same conjugating system may be present in beech and spruce acid-soluble and modified lignins. Further, these spectra are similar to the spectra usually assigned to lignin preparations of higher carbon and methoxyl contents.

## Experimental

Samples of European beech (*Fagus sylvatica*) and Sitka spruce (*Picea sitchensis*), taken from the batches prepared at the commencement of this series, were exhaustively extracted with alcohol–benzene (1 : 2), thoroughly washed with warm water, and air-dried to a moisture content of about 12%. For the lignin determination some 50 g. were oven-dried at 105°, then cooled in a desiccator. To 20 g. of this material, 125 ml. of 72% (w/w) sulphuric acid at 10° were added and the slurry was stirred and placed in a desiccator at 10° ± 0·5° for 16 hours (5 hours are sufficient for beech wood or 6 hours for spruce, but 16 hours, *i.e.*, overnight, were convenient). The mixture was then diluted with 4·750 l. of water, and the resultant 3% acid solution was gently boiled for 2 hours under reflux and filtered through a 17 G.4 sintered-glass filter to remove all sulphuric acid lignin. The volume of the filtrate and washings was measured and its ultra-violet absorption spectrum and total reducing sugar content determined.

*Isolation of Acid-soluble Lignin.*—An aliquot portion (3 l.) of the foregoing solution was passed, at a rate of about 3 l. an hour, through a column (1½ × 18 in.) containing 1 lb. of Zeo-Karb 215 which had been previously treated with 5% hydrochloric acid, distilled water (until neutral), 95% ethyl alcohol (about 3 l.; during this treatment the column was left 24 hours full of alcohol), and finally 2—3 l. of distilled water until no alcohol remained on the column. The ultra-violet absorption spectrum of the eluate, compared with the original spectrum, showed a decrease of some 90% in absorption intensity at 277·5 mμ although there was no great change in absorption at 225 mμ. Total reducing sugar determinations (W. G. Campbell and I. R. C. McDonald) indicated that no reducing sugars had been retained on the column together with the acid-soluble material. The column was then washed with 5% hydrochloric acid, distilled water until neutral, and finally 95% ethyl alcohol. Some material was immediately eluted with alcohol (total solids in a yield of 2·2% of the original wood in the case of beech and 1·0% in the case of spruce) but the greater part was eluted only after the alcohol had been in contact with the column for 16 hours (yield, 5·2% for beech and 4·8% for spruce). The total recovered material on an original wood basis was thus 7·4% for beech and 5·8% for spruce. These total solid residues were, however, resinous materials insoluble in acids, alkalis, or organic solvents.

To prepare the powder forms these aqueous-alcohol eluates were distilled in a vacuum at low temperature (≯30°) and the residue dried by azeotropic distillation with benzene or ethyl methyl ketone. From either wood an amber–brown resinous material resulted which was extremely soluble in alcohol. This was dissolved in the minimum quantity of alcohol or dioxan at room temperature (warming causes immediate polymerisation) and added to a large excess of dry ether. A buff to pale brown precipitate formed immediately in the case of beech acid-soluble lignin and a pink to pale brown precipitate in the case of spruce. A proportion of the material, however, dissolved in the alcohol–ether and was recovered by concentration of this solution and reprecipitation. These precipitates can be easily filtered off and on careful vacuum-drying are isolated as pale brown powders, readily soluble in 85% alcohol. Friction converts

either powder into transparent amber-coloured wax-like resins which are both brittle and soft. Warming converts these substances into the resinous forms which are virtually insoluble in organic solvents, acids, or alkalis.

The same technique of isolation was applied to the lignin hydrolysates of partly delignified beech and spruce wood residues. From the lignin hydrolysate of a beech wood residue (yield, 71·9% containing 0·7% of sulphuric acid lignin), 5% of beech modified lignin was isolated as a resin. From the lignin hydrolysate of a spruce wood residue (93·4% yield containing 19·0% of sulphuric acid lignin), 6·1% of spruce modified lignin was isolated as a resin. Beech modified lignin was also recovered as a pink powder, and spruce modified lignin as a buff-coloured powder. Modified lignins are not as labile as acid-soluble lignins, but resinify when heated.

Analyses, together with those of the sulphuric acid lignins, are reported in the Table.

| | Beech | | | | Spruce | | | |
|---|---|---|---|---|---|---|---|---|
| | C, % | H, % | OMe, % | Cl, % | C, % | H, % | OMe, % | Cl, % |
| Acid lignin from wood ..... | 60·4 | 5·5 | 20·0 | 0 | 63·6 | 5·7 | 14·9 | 0 |
| Acid-sol. lignin (powder) ..... | 54·0 | 5·7 | 16·4 | 0 | 46·3 | 5·5 | 3·0 | 0 |
| ,, ,, (resin) ..... | 53·9 | 5·8 | — | 0 | 54·0 | 5·3 | — | 0 |
| Modified lignin ..... | 50·4 | 5·7 | 14·6 | 6·5 | 48·7 | 5·4 | — | 5·1 |
| $C_{40}H_{43}O_{20}(OMe)_5.2HCl$ requires ... | 50·4 | 5·6 | 14·6 | 6·6 | | | | |

*Ultra-violet Spectra.*—The ultra-violet absorption spectra of the acid hydrolysates were determined in 1-cm. quartz cells in a Unicam SP 500 quartz spectrophotometer. It was necessary to dilute a normal hydrolysate 5 times to a concentration of approx. 0·04% of wood substance. The acid-soluble or modified lignins were dissolved in 80% ethyl alcohol (*c*, 3·00 mg. per 100 ml. of solution), and the spectra measured in 1-cm. quartz cells.

This paper was written after the death of Mr. W. G. Campbell. Thanks are offered to Dr. R. H. Farmer of this Laboratory who kindly assisted in its preparation. The work forms part of the current research programme of the Forest Products Research Board and is offered for publication by permission of the Department of Scientific and Industrial Research.

Section of Chemistry, Forest Products Research Laboratory,
Princes Risborough, Aylesbury, Bucks. [*Received, April 16th*, 1952.]

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## 609. *The Chemistry of the Wood Cell Wall. Part III.* The Isolation of Beech Hemicellulose A.*

By I. R. C. McDonald.

Beech hemicellulose A has been isolated from European beech wood (*Fagus sylvatica*) by a simple method in a yield of 8·2% without first delignifying the wood. The preparation was substantially free from sulphuric acid lignin and acid-soluble lignin, and had a total polysaccharide content of 91·1% (expressed as xylose). Analyses of the original extractive-free wood, the sodium hydroxide extracted wood, and the preparation of beech hemicellulose A are included.

"Hemicellulose" describes that fraction of the extractive-free wood cell wall which is soluble in dilute aqueous sodium hydroxide and is hydrolysed by hot dilute acids to reducing sugars and uronic acids. The hemicellulose complex has been subdivided further into "hemicellulose A and B etc.," and there are different definitions of these fractions. In this paper "hemicellulose A" is that fraction of the hemicellulose complex which is precipitated from the alkaline liquors by the addition of acetic acid (O'Dwyer, *Biochem. J.*, 1926, **20**, 56). For convenience, the components, pentosans, yielding furfuraldehyde have been calculated as xylans.

It has been realised that aqueous alkali dissolves part of the lignin complex as well as "hemicellulose," and the isolation of the latter in high yield from a solution containing dissolved lignin is tedious and difficult. Wise *et al.* (*Paper Trade J.*, 1946, **122**, 35) therefore

* Part II, preceding paper.

suggested that the so-called " holocellulose " fraction of wood would be a satisfactory source of " hemicellulose " because its acid lignin content was low (about 1·5%). This suggestion has been widely adopted and the usual method of preparing hemicellulose consists in delignifying a wood sample with acidified sodium chlorite and then treating the holocellulose with aqueous alkali. This alkaline extract is treated with Benedict's solution (Chanda, Hirst, and Percival, *J.*, 1951, 1240) or Fehling's solution (Yundt, *Tech. Assoc. Pulp Paper Ind.*, 1951, **34**, 89), and a copper hemicellulose complex precipitated. The complex is decomposed by hydrochloric acid, and any residual copper carefully removed. While some authors have reported that a lignin-free preparation has been obtained by this procedure, Yamamori and Tachi (*Mem. Coll. Agric. Kyoto University*, 1950, **57**, 12) stated that their preparation from elm wood (*Ulmus Davidana* Planch var. *japonica* Nakai) contained 3—3·5% of acid lignin and that repeating this method of purification six times only decreased the acid lignin content to 2·1%.

Timell and Jahn (*Svensk Papperstidning*, 1951, **24**, 831) have proved that during the chlorite delignification of paper birch the degree of polymerisation of the polysaccharide fraction is reduced, presumably by chain-length degradation. Jayme and Hanke (*Cellulosechem.*, 1943, **21**, 127) and Bublitz (*Tech. Assoc. Pulp Paper Ind.*, 1951, **34**, 427) have shown that polysaccharides are present in the chlorite delignification liquors of spruce wood and the latter worker has identified the sugars formed on hydrolysis of these substances; xylose and uronic acids have been found. It would therefore appear inadvisable to prepare hemicelluloses for structural studies from the " holocellulose " fraction and it would be preferable, if possible, to isolate the hemicellulose by direct extraction at low temperature with aqueous sodium hydroxide of minimum strength.

Table 1 lists the percentage loss of wood substance against the time of extraction of

TABLE 1. *Percentage loss of wood substance on extraction at room temperature.*

| Time, hours | 2 | 16 | 23 | 40 | 48 | 64 | 88 | 112 |
|---|---|---|---|---|---|---|---|---|
| N-NaOH | 14·1 | 18·0 | 18·7 | 18·9 | 18·8 | 19·0 | — | 20·0 |
| 0·1N-NaOH | — | 5·3 | — | — | 5·6 | 5·9 | 6·0 | — |
| 0·01N-NaOH | — | 1·6 | — | — | 1·6 | 1·6 | 1·6 | — |

TABLE 2. *Effect of varying strengths of aqueous sodium hydroxide on extracted beech wood. Extraction time, 48 hours at room temperature (results calculated as percentages of original extractive-free wood).*

| NaOH strength, N | Ratio NaOH/wood | Residue | Pentosan * | Acid lignin | Total loss | Pentosan * loss | Acid-lignin loss |
|---|---|---|---|---|---|---|---|
| — | — | 100·0 | 25·2 | 24·9 | — | — | — |
| 0·01 | 0·004 | 98·4 | 25·2 | 23·5 | 1·6 | — | 1·4 |
| 0·1 | 0·04 | 94·4 | 25·0 | 22·1 | 5·6 | 0·2 | 2·8 |
| 0·25 | 0·1 | 90·8 | 23·2 | 21·4 | 9·2 | 2·0 | 3·5 |
| 0·5 | 0·2 | 87·0 | 20·4 | 21·1 | 13·0 | 4·8 | 3·8 |
| 1 | 0·4 | 81·2 | 14·8 | 21·6 | 18·8 | 10·4 | 3·3 |
| 1·5 | 0·6 | 82·1 | 16·3 | 21·4 | 17·9 | 8·9 | 3·5 |
| 2 | 0·8 | 84·6 | 18·8 | — | 15·4 | 6·4 | — |
| 3 | 1·2 | 84·7 | 18·5 | 21·9 | 15·3 | 6·7 | 3·0 |
| 4 | 1·6 | 84·3 | — | — | 15·7 | — | — |
| 5 | 2·0 | 85·9 | 19·2 | 21·2 | 14·1 | 6·0 | 3·7 |
| 6 | 2·4 | 85·2 | — | — | 14·8 | — | — |
| 7 | 2·8 | 85·4 | 19·2 | 21·2 | 14·6 | 6·0 | 3·7 |

* The fraction, pentosan, yielding furfuraldehyde has been calculated as xylan.

beech sawdust, at room temperature, N-, 0·1N-, and 0·01N-aqueous sodium hydroxide being used. For future work an extraction time of 48 hours was adopted.

Wood samples were separately extracted for 48 hours at room temperature with varying strengths of alkaline solution. The residues were weighed and their pentosan and sulphuric acid lignin contents determined (Table 2). Increase of alkali concentration from 0·01N to N caused an increase in the amount of wood substance dissolved but the maximum was reached at the latter concentration. Further increases in alkali strength to 7N resulted in a decrease in the amount of alkali-soluble material. This variation is caused by the amount

of pentosan dissolved, because the loss of sulphuric acid lignin is approximately constant above an alkali strength of 0·1N. At and below this concentration there was no significant loss of pentosan, although up to 5·6% of wood substance had been dissolved. Also, determinations of the total reducing sugar did not show any measurable loss of total polysaccharides. These observations suggested that treating beech wood with 0·1N-sodium hydroxide should remove the major part of the soluble lignin impurities without affecting the main hemicellulose fraction.

Beech wood was therefore extracted first with 0·1N-sodium hydroxide, and the wood residue was filtered off, washed until it was neutral with distilled water, and air dried. This residue was then extracted with N-sodium hydroxide, which dissolved 13·2% of wood substance. The major part of the soluble material (11·7%) was pentosan (9·7%) and uronic anhydride (2·0%).

These analyses of the extractive-free wood and the wood after extraction with sodium hydroxide enable the individual losses sustained by the various fractions to be summed, and this total approximates to the actual measured loss. Table 3 also shows that extraction

TABLE 3. *Analyses of extractive-free beech wood and alkali-extracted wood (results expressed as percentages on extractive-free wood basis).*

| | Extractive-free wood | After treatment with 0·1N-NaOH | After treatment with 0·1N- and then N-NaOH |
|---|---|---|---|
| Yield | — | 94·4 | 81·2 |
| Sulphuric acid lignin | 24·9 | 22·1 | 21·6 |
| Methoxyl in acid lignin | 4·9 | — | 4·0 |
| ,, ,, (own basis) | 20·0 | — | 18·7 |
| Total methoxyl | 6·2 | — | 4·6 |
| Cross and Bevan cellulose | 58·1 | — | 53·7 |
| Pentosan * in Cross and Bevan cellulose | 16·3 | — | 13·5 |
| Pentosan * not in Cross and Bevan cellulose | 8·9 | — | 1·3 |
| Total pentosan * | 25·2 | 25·0 | 14·8 |
| ,, corrected for uronic anhydride | 23·8 | — | 14·1 |
| Uronic anhydride ($4 \times CO_2$ yield, corrected for xylan) | 4·2 | — | 2·2 |
| Total polysaccharide (from reducing sugar estimation) | 65·5 | 65·4 | 53·1 |
| Acid-soluble lignin (from ultra-violet spectra) | 7·5 | — | 5·3 |

*Sum of individual losses on extraction with 0·1N- and N-sodium hydroxide.*

| | | | |
|---|---|---|---|
| Sulphuric acid lignin | 3·3 | Total polysaccharide | 12·4 |
| Pentosan as xylan | 9·7 | Total loss | 17·9 |
| Uronic anhydride | 2·0 | Measured loss | 18·8 |
| Hexosan | 0·7 | | |
| Acid-soluble lignin | 2·2 | | |

* Pentosan calculated as xylan.

of wood at room temperature with aqueous sodium hydroxide dissolves only about 38% of the total pentosan fraction. The amount of this fraction (10·4% of the wood) corresponds to the pentosan not in the Cross and Bevan cellulose fraction of the original wood (8·9%), and it is noticeable that in the determination of Cross and Bevan cellulose on the alkali-extracted wood there was a very small loss of pentosan (1·3%). The yield of carbon dioxide (calculated as uronic acid) after correction for the small amount of carbon dioxide evolved from a pure polysaccharide (McCready, Swenson, and Maclay, *Ind. Eng. Chem. Anal.*, 1946, **18**, 290) indicates that approximately one half of the original polyuronides present in the wood are alkali soluble, the remainder being retained in the wood residue.

Analysis of the sample of beech hemicellulose A prepared from the N-alkaline solution by precipitation with acetic acid gave the results shown in Table 4. Of the 11·7% of pentosan and uronic acid dissolved from wood which had been extracted with 0·1N-alkali, 7·5% was recovered as hemicellulose A. This yield is higher than that previously reported for beech wood (O'Dwyer, *loc. cit.*), owing perhaps to a better isolation technique. The product contained a greater percentage of pentosan and less acid lignin.

Although the yields of acid lignin and acid-soluble lignin are low the methoxyl content is

relatively high, thus confirming the view that some of the methoxyl groups present in beech wood are associated with the polysaccharide fraction, probably the fraction which yields furfuraldehyde.

TABLE 4. *Analysis of beech hemicellulose A (results expressed as percentages on own basis).*

| | | | |
|---|---|---|---|
| Methoxyl | 4·5 | Acid soluble lignin [b] | 1·0 |
| Pentosan [a] (uncorrected) | 84·8 | Total polysaccharide | 91·1 |
| ,, (corrected for uronic anhydride) | 81·4 | Ash, as $Na_2O$ | 1·56 |
| Uronic anhydride (4 × $CO_2$ yield, corrected for pentosan) | 10·4 | $[\alpha]_D$ ($l = 2$; $c = 0{\cdot}4715$ in N-NaOH) | −90·6° |
| Sulphuric acid lignin | 0·9 | Yield, on wood basis | 8·2 |

[a] Pentosan calculated as xylan.

[b] Value determined by comparison of the specific absorption coefficients at 227·5 mμ and 225 mμ with those for beech acid-soluble lignin; solutions in N-NaOH being examined in a Unicam S.P. 500 spectrophotometer.

Chromatographic analyses of the acid-lignin hydrolysate of beech hemicellulose A showed the presence of xylose and an unidentified uronic acid which was not glucuronic acid. No other pentose or hexose was detected. For this reason the furfuraldehyde-yielding materials have been expressed as pentosan calculated as xylan.

## EXPERIMENTAL

A sample from the batch of sawdust (20—60 mesh), prepared at the start of this series of experiments from European beech (*Fagus sylvatica*), was exhaustively extracted with alcohol–benzene (approximately 1 : 2), washed with warm water until free from solvents, and air-dried to a moisture content of about 12%. In the experiments, conducted at room temperature, to determine the optimal reaction time and strength of aqueous alkali, samples (10 g.) of this extracted sawdust were placed in 250-ml. conical flasks, alkali (100 ml.) of appropriate strength was added, and the mixture was stirred slightly to ensure complete wetting of the wood. At predetermined times the samples were filtered off on 17G2 sintered-glass filters, washed well with water until neutral, dried at 105°, and weighed.

*Preparation of Bulk Sample of Beech Hemicellulose A.*—Air-dried extracted wood (500 g.) and 0·1N-sodium hydroxide (4·4 l.) were set aside for 48 hours, the mixture then filtered, and the wood residue washed with distilled water (at least 20 l., or until neutral) before being air dried. Subsequent extraction with N-sodium hydroxide was only really efficient if at least 5% of wood substance had been removed, as a deep red solution, by the 0·1N-alkali. The air-dried residue was agitated with N-sodium hydroxide (4·2 l.) and the mixture set aside for 48 hours. No special precautions, such as a nitrogen atmosphere, were necessary. The alkaline solution, which was deep yellow to pale brown, was filtered, and the residue washed. The filtrate was just acidified with acetic acid, and the crude hemicellulose precipitate filtered off overnight on a double thickness of Whatman No. 1 filter paper supported by large 60° filter funnels. This method of filtration was superior to use of sintered-glass funnels or to centrifugal methods. The crude precipitate, which should be only slightly brown, was redissolved in sodium hydroxide and precipitated by the addition of an approximately equal volume of ethyl alcohol (95%). Although this procedure converts the uronic acids into sodium salts, which might be undesirable, it renders possible a separation of the hemicellulose A from the acid-soluble lignin, which is very soluble in N-sodium hydroxide and alcohol. Three repetitions of this process gave a white product (8·2%, on the original wood basis) which was analysed by the standard methods.

*Examination of Sugars on Hydrolysis.*—Paper chromatograms of the sulphuric acid lignin hydrolysate, butanol–ethanol–water (1 : 4 : 5) being used, indicated the presence of xylose and a uronic acid, which was not glucuronic acid.

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## 610. *Synthesis of Fluoranthenes. Part X.* 10-Methylfluoranthene. Correction of its Melting Point.*

By S. Horwood Tucker and (Miss) Margaret Whalley.

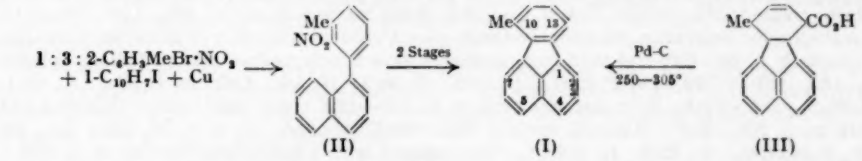
Two additional syntheses of 10-methylfluoranthene have shown that the original synthesis by Tucker and Whalley (*J.*, 1949, 3213) was sound, but that the material isolated was contaminated with a by-product. We are now in substantial agreement with Kloetzel and Mertel (*J. Amer. Chem. Soc.*, 1950, **72**, 4786).

The original synthesis of 10-methylfluoranthene (Tucker and Whalley, *loc. cit.*) gave a low overall yield, and was tedious since the starting material, 2-bromo-3-nitrotoluene had to be synthesised from *o*-toluidine by a method which involved separation of the 3-, 4-, and 5-nitro-derivatives of *o*-toluidine (Gabriel and Thieme, *Ber.*, 1919, **52**, 1079; McGookin and Swift, *J. Soc. Chem. Ind.*, 1939, **58**, 152; cf. Govaert, *Bull. Soc. chim. Belg.*, 1929, **38**, 372). Separation of pure 2-amino-3-nitrotoluene was, however, accomplished in good yield, and this by the Sandmeyer reaction gave 2-bromo-3-nitrotoluene. Even if the 2-amino-3-nitrotoluene had been contaminated with the corresponding 4- and 5-nitro-isomers, the 2-bromo-4-nitrotoluene would not have reacted with 1-iodonaphthalene, and the corresponding 5-nitro-isomer if sufficiently active to combine with 1-iodonaphthalene could not have given a cyclisable product.

Repetition of our work gave indeed the product (m. p. 105—108°) alleged to be 10-methylfluoranthene; but we now discover that this product was contaminated with a small amount of 1-(2-methyl-6-hydroxyphenyl)naphthalene. After removal of this by chromatography we obtained genuine 10-methylfluoranthene, m. p. 132—133·5°. Kloetzel and Mertel (*loc. cit.*) reported m. p. 136—137°, but Professor Kloetzel (private communication) has revised this to m. p. 135—135·5°. A sample which he kindly supplied had, by our handling, alone or mixed with our sample, m. p. 132—133·5°.

The ultra-violet absorption spectra of the two samples of 10-methylfluoranthene (*A*) were practically identical: one graph is given and compared with that of fluoranthene (*B*).

To satisfy ourselves, we have confirmed the identity of 10-methylfluoranthene by two syntheses: (i) *o*-Nitrotoluene was mercurated, then treated with bromine to give 3-bromo-2-nitrotoluene (Burton, Hammond, and Kenner, *J.*, 1926, 1802; Longo and Pirona, *Gazzetta*, 1947, **77**, 117). The crossed Ullmann reaction with 1-iodonaphthalene gave 1-(3-methyl-2-nitrophenyl)naphthalene (II). The very low yield (16%) of this compound is unusual in this type of reaction (cf. Forrest and Tucker, *J.*, 1948, 1513; Tucker and Whalley, *J.*, 1949, 632, 3213; Stubbs and Tucker, *J.*, 1951, 2936), although it has been noted that when the bromobenzene has two *ortho*-substituents (Hawkins and Tucker, *J.*, 1950, 3286) the yields in the crossed Ullmann reaction are lowered. (ii) Sorbic acid underwent a diene synthesis with acenaphthylene to give a tetrahydro-10-methylfluoranthene-13-carboxylic acid (Deno, *J. Amer. Chem. Soc.*, 1950, **72**, 4057; Kloetzel and Mertel, *loc. cit.*, used ethyl sorbate; see also Bergmann, *Nature*, 1948, **161**, 889) in which the position of the double




(II) (I) (III)

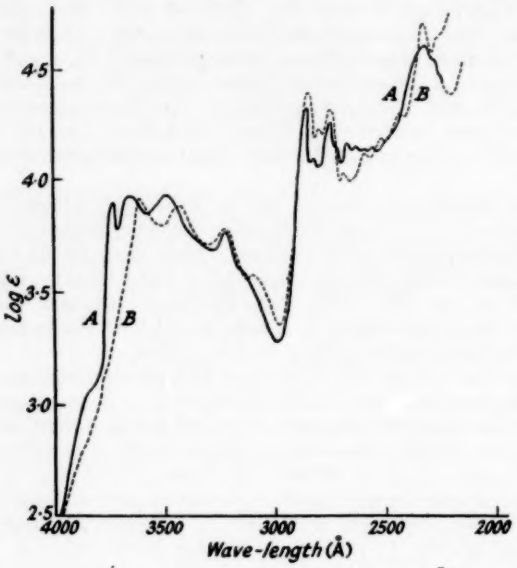
bond is unknown: it is probably not conjugated to the naphthalene ring (Deno, *loc. cit.*). It may be as indicated in (III). Heating (III) with palladium–charcoal effected dehydrogenation and decarboxylation to give 10-methylfluoranthene (cf. Wicks, Daly, and Lack, *J. Org. Chem.*, 1947, **12**, 713).

* Part IX, *J.*, 1952, 803.

Professor Kloetzel (private communication) considers the m. p. of 11-methylfluoranthene to be 90·5—91·5° (Kloetzel and Mertel, *loc. cit.*, gave 93—94°; Tucker and Whalley, *loc. cit.*, 88—90°).

## Experimental

*Repetition of Synthesis of* 10-*Methylfluoranthene* (*Tucker and Whalley*, loc. cit.).—*2-Bromo-3-nitrotoluene.* Diazotisation of 2-amino-3-nitrotoluene by Hodgson and Walker's method (*J.*, 1933, 1620) proceeded more smoothly and gave a better yield of 2-bromo-3-nitrotoluene than did that of Gibson and Johnson (*J.*, 1929, 1243). Sodium nitrite (5 g.) was added with stirring to cooled concentrated sulphuric acid (40 ml.), the temperature was raised to 70° to complete solution and then lowered to room temperature, and a solution of 2-amino-3-nitrotoluene (10 g.) in glacial acetic acid (120 ml.), cooled to room temperature, was gradually added to the nitrosylsulphuric acid solution, the temperature being kept below 20°. The solution so obtained was run into a well-stirred solution of cuprous bromide (18 g.) in 48% hydrobromic acid solution




*A*, 10-*Methylfluoranthene.*
*B*, *Fluoranthene.*

(150 ml.). Next morning, cold water (300 ml.) was carefully added. The precipitated solid was steam-distilled, to give 2-bromo-3-nitrotoluene as a pale yellow solid, m. p. 38—40° (9·5 g., 68%). This was of good enough quality for use in the crossed Ullmann reaction. Recrystallised from light petroleum (b. p. 40—60°), it had m. p. 41—42°. Completion of the synthesis of (I) gave the product, m. p. 105—108°, as described; but when this had been extracted with 10% potassium hydroxide solution (or when the material precipitated on the copper had been similarly treated) the residue formed a picrate (from methanol), m. p. 197—199°, which by chromatographic separation (alumina–benzene) gave a product whence crystallisation from light petroleum (b. p. 40—60°) and then from methanol gave faintly yellow, diamond-shaped plates, m. p. 132—133·5° [133—134·5° (corr.)] (Found: C, 94·3; H, 5·4. Calc. for $C_{17}H_{12}$: C, 94·4; H, 5·6%). The picrate, from methanol, has m. p. 197—199° [200—202° (corr.)] (Kloetzel and Mertel, m. p. 201—202°; Kloetzel, revised, 200—200·5°) (Found: C, 61·9; H, 3·3. Calc. for $C_{17}H_{12},C_6H_3O_7N_3$: C, 62·0; H, 3·4%). The complex with *s*-trinitrobenzene has m. p. 218—219°; that with 2 : 4 : 7-trinitrofluorenone, m. p. 215—216° (Found: C, 67·6; H, 3·3; N, 7·8. Calc. for $C_{17}H_{12},C_{13}H_5O_7N_3$: C, 67·8; H, 3·2; N, 7·9%). The phenolic impurity referred to above was isolated by the following modification of the cyclisation process.

1-(2-*Hydroxy*-6-*methylphenyl*)*naphthalene.* A mixture of 1-(2-amino-6-methylphenyl)naphthalene (0·78 g.), concentrated sulphuric acid (1·5 ml.), and water (10 ml.) was diazotised at 0° by addition of sodium nitrite (0·25 g.) in water (2 ml.). Excess of nitrite was destroyed by urea.

The solution was poured into a 33% solution of dimethylamine (*ca.* 1 g.) containing excess of sodium carbonate solution (Elks and Hey, *J.*, 1943, 441). The brick-red precipitate obtained was dissolved in acetic acid, and warmed whilst hydrogen chloride was passed in. Dilution with water gave an oil which was chromatographed (alumina–benzene). A blue-fluorescent band, on elution, gave 10-methylfluoranthene (I). The column was further eluted with benzene containing 5% of ethanol, and gave a solid (0·06 g.) which after crystallisation from light petroleum (b. p. 60—80°) gave very pale yellow monoclinic prisms of 1-(2-*hydroxy*-6-*methylphenyl*)*naphthalene*, m. p. 100—102° (Found: C, 87·2; H, 6·2. $C_{17}H_{14}O$ requires C, 87·2; H, 6·0%).

*Synthesis of* 10-*Methylfluoranthene* (I) *from* 1-*Iodonaphthalene and* 3-*Bromo*-2-*nitrotoluene*.—1-(3-*Methyl*-2-*nitrophenyl*)*naphthalene* (II). 1-Iodonaphthalene (2·54 g., 0·01 g.-mol.) and an excess of 3-bromo-2-nitrotoluene (b. p. 125—130°/8 mm.; 7·2 g.) and of copper bronze were heated at 240° for 7 hours. The black liquor was extracted with light petroleum (b. p. 60—80°), filtered from copper, and distilled, to remove unchanged liquids, up to 130°/8 mm. The residue was dissolved in light petroleum (b. p. 60—80°) and the solution chromatographed (alumina). The first eluate contained unchanged oils which on dilution with light petroleum [b. p. 40—60°; 10 parts (v/v)] and storage at 0° gave 1 : 1′-dinaphthyl (m. p. 156—158°). Subsequent eluates gave, even without cooling in ice, but on concentration followed by crystallisation from methanol, faintly green, long rectangular laminæ of 1-(3-*methyl*-2-*nitrophenyl*)*naphthalene* (II), m. p. 118—119° (0·43 g., 16% calc. on 1-iodonaphthalene) (Found: C, 77·5; H, 4·95; N, 5·3. $C_{17}H_{13}O_2N$ requires C, 77·6; H, 4·9; N, 5·3%). Lower yields were obtained by heating equimolecular quantities of the reactants with excess of copper at 200° for 5 hours. In one experiment at 240° a small amount of 3 : 3′-dimethyl-2 : 2′-dinitrodiphenyl, m. p. 226—227°, was obtained from a final eluate of the column with acetone (cf. Longo and Pirona, *loc. cit.*).

1-(2-*Amino*-3-*methylphenyl*)*naphthalene*. The above nitro-compound (II) (0·36 g.) with hydrogen and Raney nickel in ethanol gave theoretical absorption of hydrogen (20 minutes), and the resulting 1-(2-*amino*-3-*methylphenyl*)*naphthalene* crystallised from light petroleum (b. p. 60—80°) and then from methanol in short pointed lath-like crystals, m. p. 109—110° (0·23 g., 73%) (Found: C, 87·4; H, 6·4; N, 6·1. $C_{17}H_{15}N$ requires C, 87·5; H, 6·4; N, 6·0%). Reduction with hydrogen and 10% palladium–charcoal was very rapid.

10-*Methylfluoranthene* (I). The above amine (0·22 g.) in glacial acetic acid (2 ml.) was poured into a solution of sodium nitrite (0·069 g.) in acetic acid (1·5 ml.) and concentrated sulphuric acid (0·3 ml.) kept in ice. The clear yellow solution was diluted with water, and warmed on the steam-bath. Yellow crystals separated, and when the liquor became colourless (*ca.* 10 minutes) the whole was boiled, cooled, and extracted with ether, the ethereal extract was shaken with 10% potassium hydroxide solution until the alkaline extract was colourless, and then washed with water, and after removal of ether the residue was crystallised from light petroleum (b. p. 40—60°) and then from methanol to give pale yellow diamond-shaped plates (0·09 g., 45%) of 10-methylfluoranthene (I) (m. p. 132—133°). Addition of copper bronze had no facilitating influence on the cyclisation.

*Synthesis of* 10-*Methylfluoranthene* (I) *from Acenaphthylene and Sorbic Acid*.—9 : 10 : 13 : 14(?)-Tetrahydro-10-methylfluoranthene-13-carboxylic acid (0·185 g.) (Deno, *loc. cit.*) was heated at 250—270° with 10% palladium–charcoal (0·12 g.) in a current of carbon dioxide, the hydrogen evolved (in 40 minutes, *ca.* 25 ml.; theor., 31 ml.) being measured in an azotometer over 50% potassium hydroxide solution (Linstead, *J.*, 1937, 1153). Further heating for a short time at 305° caused frothing probably owing to evolution of carbon dioxide from the carboxylic acid since no evolution of hydrogen took place. A sublimate, formed on the sides of the tube, and the residue, on extraction with light petroleum (b. p. 40—60°), gave 10-methylfluoranthene (I).

*Ultra-violet Absorption Spectrum of* 10-*Methylfluoranthene*.—This was recorded by the Unicam spectrophotometer, with ethanol as solvent: maxima occurred at (λ in Å) p-bands 3740 (log ε 3·90), 3660 (3·93), 3500 (3·94), 3240 (3·77); β-bands 2880 (4·34), 2770 (4·28), 2690 (4·18), 2650 (4·16), 2570 (4·17); β′-band 2350 (4·64).

We are indebted to Mr. W. C. Russell for the preparation of 2-bromo-3-nitrotoluene, to Mr. A. J. N. Hope, B.Sc., for recording the absorption spectrum of 10-methylfluoranthene, and to Miss M. W. Christie for micro-analyses.

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## 611. *High-polymer Solutions. Part I. Osmotic-pressure Measurements with Poly(vinyl alcohol) Membranes.*

By H. T. Hookway and R. Townsend.

The behaviour of poly(vinyl alcohol) films as semipermeable membranes in non-aqueous systems has been examined. Membranes with different degrees of semipermeability have been prepared by varying the original water content (O.W.C.) of the films before conditioning them to the solvent. The permeability of the films decreases with increasing O.W.C. By using a membrane of O.W.C. 0·071 g. per g. of poly(vinyl alcohol) it has been possible to measure polymer molecular weights from $\overline{M}_n \sim 1 \times 10^6$ down to 2000; in no case was there evidence of solute diffusion through the film.

With the polymer–solvent systems studied it has been found that the relationship between $\pi$ and $C$ is best expressed by the equation $\pi/C = a + bC$ ($\pi$ = osmotic pressure, $C$ = concentration, $a$ and $b$ = constants).

The reproducible and accurate determination of number average molecular weights by osmotic methods depends to a very large extent on the use of carefully prepared and truly semipermeable membranes. With solutions of many polymers which have low molecular weights or are heterodisperse, it has hitherto proved impossible to achieve complete retention of the solute without reducing the rate of passage of solvent through the membrane to negligible values.

The most widely used semipermeable membranes are based on gel cellophane or denitrated collodion. Melville and his co-workers (*Nature*, 1946, **157**, 74) have also used bacterial cellulose films. The lower limit of semipermeability for denitrated collodion membranes in non-aqueous systems is $\overline{M}_n \sim 30{,}000$—$40{,}000$ (Flory, *J. Amer. Chem. Soc.*, 1943, **65**, 372) and for gel cellophane ~10,000 (Wagner, *Ind. Eng. Chem.*, 1944, **16**, 520). Philipp and Bjork (*J. Polymer Sci.*, 1951, **6**, 383) employed an old, slow, and much used gel cellophane membrane to evaluate a cellulose acetate fraction of $\overline{M}_n$ 1450 (cryoscopically measured), but it was necessary to correct for solute diffusion. For this fraction, normal gel cellophane membranes were far too permeable for use.

By using the poly(vinyl alcohol) membranes described below it has been possible to make accurate measurements of the molecular weights in non-aqueous solutions of polymers from $\overline{M}_n \sim 2000$ upwards. The rate of solvent transfer across these membranes is sufficiently rapid to enable simple unsymmetrical osmometers to be used, equilibrium being reached in 2—3 hours.

### Experimental.

Measurements of osmotic pressure were made in modified Zimm–Meyerson osmometers (*J. Amer. Chem. Soc.*, 1946, **68**, 911). A central tension-adjusting screw was used to clamp the plates in place of the original spring-loaded wing nuts. Precision-bore tubing was used for the measuring capillary (int. diam. 0·05 cm.), reference capillary (int. diam. 0·05 cm.), and filling capillary (int. diam. 0·2 cm.). In order to reduce distortion, the measuring capillary was not supported by the filling tube; the reference capillary was attached to the filling tube. The glass cell was 1·0 cm. deep and of 2·0-cm. internal diameter. The filling capillary was closed by a metal rod with a mercury seal, the rod being used to adjust the meniscus levels of the solutions. The glass cylinder containing osmometer and pure solvent was closed with a well-fitting cap.

The constant-temperature water-bath was kept in a draught-free room at 25° ± 1°. During 2—3 hours, temperature control in the bath was ~±0·001°; 31·5° ± 0·002° could be maintained for long periods. Despite the unsymmetrical nature of the osmometers, the combination of good temperature control and fast membranes resulted in stable osmotic heads with no sign of oscillation about an equilibrium position.

*Poly(vinyl alcohol) Membranes.*—The poly(vinyl alcohol) used was prepared from medium viscosity poly(vinyl acetate) and contained about 1% of residual acetate. Membranes were

prepared by evaporation of an aqueous (1·5% w/v) solution of poly(vinyl alcohol) at 26° ± 0·1° and 40% relative humidity. Their normal thickness was 0·002—0·003 in. Before use, the membranes were conditioned to atmospheres of known relative humidity for 5 days and their water contents then estimated by displacement of the water with dry alcohol and titration of the wet alcohol with the Karl Fischer reagent.

Typical membranes were dried over phosphoric oxide to constant weight, it then being found that no water could be displaced with dry alcohol. Membranes which had been dried in this way, weighed, and then stored at different relative humidities were found to have a weight increase equal, within experimental error, to the weight of water removed by dry alcohol (Table 1).

TABLE 1. *Water content of poly(vinyl alcohol) membranes.*

| Relative humidity (%) | Wt. of dry membrane (g.) | Wt. of $H_2O$ absorbed (g.) | Wt. of $H_2O$ displaced by dry alcohol (g.) | O.W.C. (g./g.) |
|---|---|---|---|---|
| 0 | 0·3130 | — | 0·000 | 0·000 |
| 32 | 0·3106 | 0·0049 | 0·005 | 0·015 |
| 40 | 0·3462 | 0·0075 | 0·007 | 0·022 |
| 44 | 0·3501 | 0·0088 | 0·009 | 0·025 |
| 70 | 0·3034 | 0·0217 | 0·022 | 0·071 |
| 75 | 0·3130 | 0·0270 | 0·027 | 0·086 |
| 93 | 0·3687 | 0·1169 | 0·117 | 0·317 |

After dehydration by alcohol, the membranes were conditioned to benzene by immersion in 1 : 1 alcohol–benzene for 2 hours and then in benzene for 3 hours. The membranes were installed in the osmometers, and their "speeds" to benzene measured at 31·5° ± 0·002° by observing the fall of the liquid meniscus in the measuring capillary. Fig. 1 shows the relation between original water content of membranes [O.W.C.; stated throughout in g. of water per g. of poly(vinyl alcohol)] and speed to benzene. In these experiments the fall of the benzene meniscus did not follow the simple exponential relation used by French and Ewart (*Ind. Eng. Chem., Anal.*, 1947, **19**, 165) to evaluate the permeability constant of their membranes. Nonetheless a family of curves was obtained such that for any two membranes at times $t_1$, $t_2$, $t_3$, etc., $(h^{I}/h^{II})_{t_1} = (h^{I}/h^{II})_{t_2}$, etc. The "speeds" recorded in Table 2 have been calculated by arbitrarily assigning a speed of 1 to the membrane of O.W.C. 0·317 g./g. and relating the other membrane speeds to this value.

TABLE 2. *Relation between O.W.C. and speed to benzene at* 31·5°.

| O.W.C. (g./g.) | 0·015 | 0·025 | 0·050 | 0·071 | 0·086 | 0·317 |
|---|---|---|---|---|---|---|
| Speed | 97 | 88 | 11·8 | 7·1 | 5·9 | 1·0 |

The most striking feature in Table 2 is that membrane speed decreases with increasing O.W.C., in complete contrast to the behaviour of bacterial cellulose under the same conditions (authors, unpublished work) or of gel cellophane (Carter and Record, *J.*, 1939, 660) and bacterial cellulose (Melville and Masson, *J. Polymer Sci.*, 1949, **4**, 323) on swelling in alcohol–water. Semipermeability towards benzene solutions of polystyrene of $\overline{M}_n$ ~200,000 was shown with membranes possessing an O.W.C. of 0·05 g./g., and equilibrium osmotic heads were obtained with membranes of O.W.C. 0·06 g./g. The work described subsequently in this paper was carried out with a membrane of O.W.C. 0·071 g./g. With this membrane, and the osmometers described above, an osmotic head of 1 cm. is developed in about 2 hours.

*Materials.*—Benzene, butanone, and toluene were purified by drying and careful fractionation. One particularly pure sample of benzene was obtained by distillation, treatment with mercuric acetate, re-distillation, and repeated crystallisation. The densities and refractive indices of these materials were in excellent agreement with accepted values.

Ethyl alcohol was dried and purified by Smith's method (*J.*, 1927, 1288).

*Preparation of Polymer Solutions.*—All solutions were made up by weight. A stock solution of the most concentrated solution to be used was made up and portions diluted by weight to give the lower concentrations.

*Measurement of Osmotic Head.*—The head developed by the most dilute solution was measured first, then those by the other solutions in order of increasing concentration. The cells were washed 3—6 times with solution, filled with solution, and placed in the solvent container which was already in the thermostat bath. Equilibrium was then allowed to be established, a static method of measurement being used. The change of concentration due to the small amount of

solvent which moved through the membrane in establishing a head of 1 cm. was about 0·06%. When osmotic heads in excess of 2 cm. were measured, the expected equilibrium position was roughly determined and the solution meniscus then set about 1 cm. below this position. An osmometer was not considered satisfactory if, with solvent on both sides of the membrane, the difference in level between measuring and reference capillary menisci exceeded $\pm$0·001 cm. The speed of the membranes to pure solvent and the heights of solvent in the measuring and the reference capillary were checked at the beginning and end of each set of measurements. The stability of the equilibrium osmotic heads was usually observed during 24 hours for at least 3 of the concentrations measured.

## Results.

In order to establish the precision of the experimental technique, osmotic pressure measurements were made on an unfractionated emulsion of polystyrene in benzene, toluene, and butanone solutions. The graphs of $\pi/C$ against $C$ (concentration) are shown in Fig. 2. The concentration units have been converted into g./100 c.c. of solution at 31·5°, by using values of 0·8665, 0·8552,

Fig. 1. *Relation between O.W.C. and speed to benzene.*

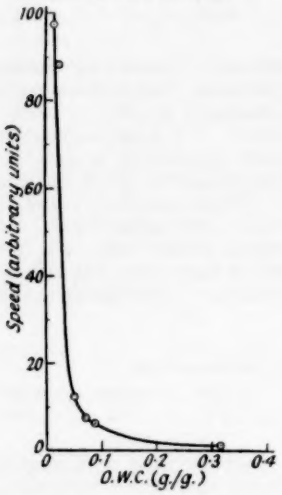
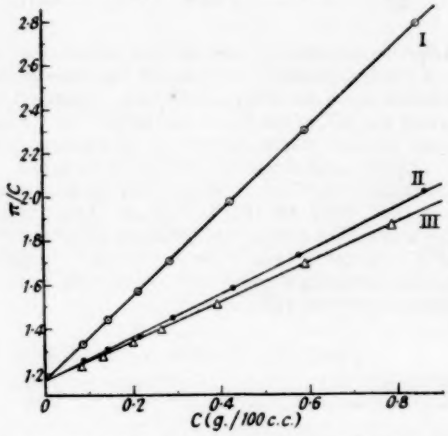



Fig. 2. *Variation of $\pi/C$ with $C$ for polystyrene in (I) toluene, (II) benzene, (III) butanone.*




$\lim (\pi/C)_{c \to 0} = 1{\cdot}170$; $\overline{M}_n = 220{,}700$.

0·7896 g./c.c. for the densities of benzene, toluene, and butanone respectively and 1·08 g./c.c. for polystyrene (cf. Boyer and Spencer, *J. Polymer Sci.*, 1948, **3**, 125).

The osmotic pressures ($\pi$) were calculated from the relation :

$$\pi = (h_s - h_0)\rho_s \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where $h_0$ is the capillary rise in the reference capillary, $h_s$ is the combined osmotic and capillary rise in the measuring capillary, and $\rho_s$ is the density of the polymer solution. The difference between the surface tension of the pure solvents and that of the most concentrated solutions of the polymer was shown to be negligible. Strictly, equation (1) does not yield the true osmotic pressure since the reference level should not be that of pure solvent in the outer container, but rather the point at which true osmotic equilibrium obtains (Lang, *Kolloid Z.*, 1951, **122**, 165; Gilbert, Graff-Baker, and Greenwood, *J. Polymer Sci.*, 1951, **6**, 585). With the Zimm–Meyerson osmometer, equilibrium will obtain at the centre of the vertical membranes. Thus, if $h$ is the depth of immersion of the membrane centre,

$$\pi^* = (h_s + h)\rho_s - (h_0 + h)\rho_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where $\rho_0$ is the density of the solvent and $\pi^*$ the true osmotic pressure.

Hence

$$\pi^* - \pi = (h_0 + h)\,(\rho_s - \rho_0) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

In our experiments, $h = 3$ cm. and $h_0$ 2—3 cm.; the error in $\pi$ introduced by using equation (1) was less than 1% at the lowest polymer concentrations.

Although it is desirable to convert the polymer weight concentrations into units of g./c.c. of solution, it is often more convenient to convert them into units of g./c.c. of pure solvent by assuming $\rho_s = \rho_0$ at all polymer concentrations and then calculating $\pi$ from equation (4), *viz.*:

$$\pi = (h_s - h_0)\rho_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

It may readily be shown that the percentage error, $E$, introduced by assuming that $\rho_s = \rho_0$ is

$$E = C(1 - \rho_0/\rho_p) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

where $C$ is the polymer concentration in g./100 g. of solution and $\rho_p$ the density of the polymer. Thus for a 1% w/w solution, if $\rho_p > \rho_0$, $E < 1\%$. For a benzene solution of polystyrene at 31·5°, $E = 0{\cdot}198\%$ when $C = 1$. All subsequent results have been presented on the basis $\rho_s = \rho_0$, and $\pi$ has been calculated by means of equation (4).

The experimental measurements have been fitted to the equation $\pi/C = a + bC$; the values of $\pi/C$, $C$, $a$, $b$, and $(\pi/C - a)/C$ are given in the tables which follow. All measurements were made at 31·5° and with a membrane of O.W.C. 0·071 g./g.

Measurements for benzene solution on a low-conversion benzoyl-peroxide-catalysed polystyrene (*A*), a freeze-dried high-conversion commercial polystyrene (*B*), and the latter material treated with propylene oxide (*C*) to remove the polymer of low molecular weight (Cleverdon and Laker, *J. Appl. Chem.*, 1951, **1**, 1) are shown in Table 3.

TABLE 3.

| Polymer | $C$ (g./100 c.c.) | $\pi$ (g./sq. cm.) | $\pi/C$ | $a$ | $b$ | $(\pi/C - a)/C$ | $\overline{M}_n$ |
|---|---|---|---|---|---|---|---|
| *A* | 0·0437 | 0·717 | 16·40 | | | 2·059 | |
| | 0·0872 | 1·438 | 16·50 | | | 2·179 | |
| | 0·1452 | 2·416 | 16·63 | 16·31 | 2·256 | 2·203 | $1{\cdot}58 \times 10^4$ |
| | 0·2185 | 3·671 | 16·80 | | | 2·242 | |
| | 0·2923 | 4·959 | 16·97 | | | 2·258 | |
| *B* | 0·0934 | 0·581 | 6·215 | | | 1·125 | |
| | 0·1559 | 0·977 | 6·267 | | | 1·007 | |
| | 0·2343 | 1·486 | 6·342 | 6·11 | 0·989 | 0·990 | $4{\cdot}19 \times 10^4$ |
| | 0·3145 | 2·015 | 6·406 | | | 0·941 | |
| | 0·4706 | 3·094 | 6·575 | | | 0·988 | |
| *C* | 0·0858 | 0·148 | 1·727 | | | 0·897 | |
| | 0·2160 | 0·398 | 1·841 | | | 0·884 | |
| | 0·2890 | 0·552 | 1·910 | 1·65 | 0·900 | 0·897 | $15{\cdot}65 \times 10^4$ |
| | 0·4310 | 0·879 | 2·039 | | | 0·902 | |
| | 0·8663 | 2·106 | 2·431 | | | 0·902 | |

The relations between $\pi/C$ and $C$ for benzene solutions of poly(methyl methacrylate) (*D*) and poly(vinyl thiolacetate) (*E*) are shown in Table 4. The poly(methyl methacrylate) was a fraction obtained from a commercial polymer and the poly(vinyl thiolacetate) had been freed from material of very low molecular weight by repeated dissolution in dioxan and precipitation with absolute alcohol.

TABLE 4.

| Polymer | $C$ (g./100 c.c.) | $\pi$ (g./sq. cm.) | $\pi/C$ | $a$ | $b$ | $(\pi/C - a)/C$ | $\overline{M}_n$ |
|---|---|---|---|---|---|---|---|
| *D* | 0·0858 | 0·165 | 1·920 | | | 0·932 | |
| | 0·1450 | 0·285 | 1·970 | | | 0·896 | |
| | 0·2176 | 0·445 | 2·043 | | | 0·892 | |
| | 0·2910 | 0·614 | 2·110 | 1·840 | 0·930 | 0·928 | $14{\cdot}11 \times 10^4$ |
| | 0·4333 | 0·974 | 2·248 | | | 0·942 | |
| | 0·5802 | 1·380 | 2·378 | | | 0·927 | |
| | 0·8700 | 2·307 | 2·652 | | | 0·933 | |
| *E* | 0·0866 | 0·232 | 2·680 | | | 1·155 | |
| | 0·1454 | 0·399 | 2·747 | | | 1·148 | |
| | 0·2165 | 0·612 | 2·825 | 2·580 | 1·134 | 1·131 | $10{\cdot}01 \times 10^4$ |
| | 0·2892 | 0·841 | 2·909 | | | 1·138 | |
| | 0·4332 | 1·329 | 3·068 | | | 1·126 | |
| | 0·6481 | 2·148 | 3·315 | | | 1·134 | |

Measurements for benzene solutions of polyacenaphthylenes of different degrees of polymerisation (*F*, *G*, *H*) are shown in Table 5.

TABLE 5.

| Polymer | $C$ (g./100 c.c.) | $\pi$ (g./sq. cm.) | $\pi/C$ | $a$ | $b$ | $(\pi/C - a)/C$ | $\overline{M}_n$ |
|---|---|---|---|---|---|---|---|
| *F* | 0·0867 | 0·598 | 6·893 | | | 1·188 | |
| | 0·1460 | 1·016 | 6·962 | | | 1·178 | |
| | 0·2173 | 1·532 | 7·050 | 6·79 | 1·200 | 1·196 | $3·8 \times 10^4$ |
| | 0·2900 | 2·070 | 7·140 | | | 1·202 | |
| | 0·4309 | 3·150 | 7·309 | | | 1·205 | |
| *G* | 0·0872 | 0·827 | 9·485 | | | 1·204 | |
| | 0·1464 | 1·399 | 9·555 | | | 1·195 | |
| | 0·2166 | 2·088 | 9·640 | 9·38 | 1·200 | 1·200 | $2·75 \times 10^4$ |
| | 0·2912 | 2·834 | 9·730 | | | 1·203 | |
| | 0·4350 | 4·306 | 9·899 | | | 1·193 | |
| *H* | 0·0436 | 0·958 | 21·95 | | | 1·147 | |
| | 0·0872 | 1·918 | 22·00 | | | 1·147 | |
| | 0·1462 | 3·225 | 22·06 | 21·90 | 1·125 | 1·094 | $1·18 \times 10^4$ |
| | 0·2175 | 4·813 | 22·14 | | | 1·103 | |
| | 0·2892 | 6·424 | 22·21 | | | 1·072 | |

Table 6 shows the relation between $\pi/C$ and $C$ for a styrene–divinylbenzene soluble copolymer (*J*) containing a nominal 0·1% of divinylbenzene and polymerised at 60° with 2% of benzoyl peroxide as catalyst. The polymer was freed from polymer of very low molecular weight by dissolution in benzene and precipitation of the polymer with methyl alcohol. The measurements were carried out on benzene solutions.

TABLE 6.

| Polymer | $C$ (g./100 c.c.) | $\pi$ (g./sq. cm.) | $\pi/C$ | $a$ | $b$ | $(\pi/C - a)/C$ | $\overline{M}_n$ |
|---|---|---|---|---|---|---|---|
| *J* | 0·2171 | 0·077 | 0·355 | | | 0·460 | |
| | 0·2887 | 0·113 | 0·390 | | | 0·371 | |
| | 0·4365 | 0·199 | 0·456 | 0·255 | 0·456 | 0·460 | $1 \times 10^6$ |
| | 0·5208 | 0·255 | 0·489 | | | 0·449 | |
| | 0·6435 | 0·354 | 0·549 | | | 0·457 | |
| | 0·8656 | 0·562 | 0·649 | | | 0·455 | |

Osmotic-pressure measurements on a phenol–formaldehyde " Novolac " fraction in butanone are summarised in Table 7.

TABLE 7.

| $C$ (g./100 c.c.) | $\pi$ (g./sq. cm.) | $\pi/C$ | $\overline{M}_n$ | $M_n$ (cryoscopic) |
|---|---|---|---|---|
| 0·0791 | 9·482 | $119·8_4$ | | |
| 0·0591 | 7·080 | 119·8 | 2156 | 2140 |
| 0·0395 | 4·734 | 119·8 | | |
| 0·0198 | 2·369 | $119·7_6$ | | |

The measurements recorded in Table 8 were made on benzene solutions of a polystyrene of low molecular weight, prepared by stannic chloride catalysis in ethylene dichloride (Pepper, *Sci. Proc. Roy. Dublin Soc.*, 1951, **25**, 239).

TABLE 8.

| $C$ (g./100 c.c.) | $\pi$ (g./sq. cm.) | $\pi/C$ | $\overline{M}_n$ | $\overline{M}_n$ (cryoscopic) |
|---|---|---|---|---|
| 0·0435 | 5·959 | 137·1 | 1900 | 1900 |
| 0·0216 | 2·954 | 136·5 | | |

## DISCUSSION

The membranes described in this paper considerably extend the scope and accuracy of osmotic-pressure measurements on polymer solutions. Since the molecular weight obtained from osmotic measurements is a number average it is of particular importance to avoid diffusion of polymer. In our view a membrane should be characterised, where possible, by measurements with a polymer whose number average molecular weight has been checked by an independent method. Stability of osmotic head and non-precipitability of polymer from the solvent side of the osmometer do not always constitute a rigid test for the diffusion of polymer of low molecular weight. Thus three independent measurements of the molecular weight of a broad fraction of a commercial polystyrene from

which polymer of low molecular weight had been (partly) removed yielded the following results. Laboratories A and B used Fuoss–Mead osmometers and denitrated collodion membranes; in both cases apparently rigorous checks were made for solute diffusion.

| Laboratory: | A | B | This work |
|---|---|---|---|
| $\overline{M}_n$ ..... | 210,000 | 222,500 | 156,500 |

Fox, Flory, and Bueche suggested recently (*J. Amer. Chem. Soc.*, 1951, **73**, 285) that, at the polymer concentrations normally used in osmotic measurements of molecular weight, the curvature of the $\pi/C$ against $C$ plot is sufficiently marked to require its extrapolation to zero concentration as a curve rather than as a straight line. It will be apparent that with none of the heterodisperse polymers investigated in this work is there any justification for other than a rectilinear extrapolation. A statistical justification of this view will be the subject of a further communication. It appears that, whilst the ultimate form of the $\pi/C$ against $C$ relationship may well be curvilinear, the errors introduced in a general application of the Flory equation to heterodisperse polymers may be greater than those encountered experimentally (cf. Cleverdon and Laker, *Chem. and Ind.*, 1951, 272).

This work forms part of the programme of the Chemical Research Laboratory and the subject matter forms part of B.P. Appl. 29,620/51. This paper is published by permission of the Director. The authors thank Mr. R. W. Hall for permission to quote the cryoscopic measurements on the " Novolac " fraction, Dr. D. C. Pepper for a gift of polystyrene of low molecular weight, the Distillers Co. Ltd. for gifts of polystyrene, and Imperial Chemical Industries Limited, Plastics Division, for a gift of poly(methyl methacrylate).

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## 612. *The Conversion of Silver (+)-2-Ethylhexanoate into (+)-3-Bromoheptane.*

By D. C. ABBOTT and C. L. ARCUS.

The reaction of bromine with silver (+)-2-ethylhexanoate, from which previous investigators have obtained (±)-3-bromoheptane, has been found to yield (+)-3-bromoheptane when the silver bromide formed (a catalyst for bromine exchange) is removed at an early stage.

Attempts to repeat the conversion of silver α-phenylpropionate into 1 phenylethyl bromide (Arcus, Campbell, and Kenyon, *J.*, 1949, 1510) have not given satisfactory yields of the bromide; 1-phenylethyl α-phenylpropionate has been isolated from the products of reaction.

THE conversion of the silver salt of a carboxylic acid to an alkyl bromide by reaction with two equivalents of bromine in carbon tetrachloride has been carried out with the silver salts

$$R{\cdot}CO_2Ag + Br_2 \longrightarrow R{\cdot}CO{\cdot}OBr + AgBr \quad . \quad . \quad . \quad . \quad . \quad (1)$$

$$R{\cdot}CO{\cdot}OBr \longrightarrow RBr + CO_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

of several optically active acids. The formation of (+)-1-phenylethyl bromide by the reaction of bromine with silver (+)-α-phenylpropionate has been described (Arcus, Campbell, and Kenyon, *J.*, 1949, 1510). Bell and Smyth (*ibid.*, p. 2372) allowed bromine to react, with cooling, with silver (+)- and (−)-2-ethylhexanoate and, after 15 minutes' heating, obtained (±)-3-bromoheptane from each reaction. [Arnold and Morgan (*J. Amer. Chem. Soc.*, 1948, **70**, 4248) had earlier obtained (±)-3-bromoheptane by heating (+)- and (−)-2-ethylhexanoic acids first with silver acetate and then for 7 hours with bromine; Doering and Farber (*ibid.*, 1949, **71**, 1514) have pointed out that heating for this time with silver bromide may have racemised the 3-bromoheptane.] From silver (+)-α-benzylbutyrate Bell and Smyth obtained (−)-2-bromo-1-phenylbutane having $[\alpha]_{5461}$ −2·85° (in alcohol), but a similar experiment with silver (−)-α-benzylbutyrate gave (±)-2-bromo-1-phenylbutane.

It has been found (Arcus and Boyd, *J.*, 1951, 1580) that racemisation of (+)-1-phenylethyl bromide in solution in carbon tetrachloride is catalysed by silver bromide. Freshly prepared silver bromide effects some racemisation and the rate is greatly increased when hydrogen bromide is present. Racemisation is ascribed to bromine exchange at the surface of the silver bromide. Since bromine yields hydrogen bromide by substitution in 1-phenylethyl bromide, it was not possible to ascertain whether bromine exchange with elementary bromine takes place.

It appeared that it would be advantageous to remove the silver bromide as early as practicable and, if possible, before stage (2) commences; accordingly, silver (+)-2-ethylhexanoate was allowed to react with bromine in carbon tetrachloride, the silver bromide was removed by filtration, and the filtrate heated for completion of stage (2). Three experiments, in which the bromine was added at approximately 15°, 5°, and −15°, yielded (+)-3-bromoheptane having $\alpha_{5893}$ +0·13°, +0·15°, and +0·39° [*l* 1·0; calculated for the fully-active (+)-acid] respectively.

The retention of optical activity indicates that the 3-heptyl group cannot have an extended life as a free radical or as an ion; further information cannot be deduced because the configurational relation between (+)-2-ethylhexanoic acid and (+)-3-bromoheptane is not known.

It was intended to apply this method to the reaction with silver α-phenylpropionate but attempts, nine in all, by the original method and modifications of it failed to yield appreciable quantities of 1-phenylethyl bromide; the only identified product was 1-phenylethyl α-phenylpropionate. The ester R·$CO_2$R, which is probably formed according to the equation

$$2R{\cdot}CO_2Ag + Br_2 \longrightarrow R{\cdot}CO_2R + CO_2 + 2AgBr$$

has been reported as a product from a number of silver salt–bromine reactions (from silver butyrate, Bockemüller and Hoffmann, *Annalen*, 1935, **519**, 185; silver laurate, Lüttringhaus and Schade, *Ber.*, 1941, **74**, 1565; silver benzoate, phenylacetate, and *o*-nitrobenzoate, Oldham, *J.*, 1950, 105).

## Experimental

2-Ethylhexanoic acid was prepared and resolved by the method of Kenyon and Platt (*J.*, 1939, 633); the (+)-acid of maximum rotation had b. p. 117—119°/15 mm., $d_4^{17}$ 0·908, $n_D^{25}$ 1·4231, $[\alpha]_{5893}^{17}$ +8·89° (homogeneous), 99% optically pure by comparison with Kenyon and Platt's value.

Silver (+)- and (−)-2-ethylhexanoates were prepared, and carbon tetrachloride and bromine were dried, by methods previously described (Abbott and Arcus, *J.*, 1952, 1516).

A suspension of the silver salt (10·0 g.) of (+)-2-ethylhexanoic acid ($\alpha_{5893}^{22}$ +15·07°, *l* 2·0, 93% optically pure) in carbon tetrachloride (100 ml.) was cooled, in ice and salt at between −10° and −15°, and a solution of bromine (6·2 g.) in carbon tetrachloride (25 ml.) was added during 15 minutes with vigorous stirring. After a further 15 minutes' stirring, the reaction mixture was filtered by suction through a sintered-glass filter in a closed apparatus. The filtrate was heated under reflux for 30 minutes, cooled, washed with aqueous sodium metabisulphite (20%; 20 ml.), with aqueous sodium carbonate (20%, 25 ml.), and with water, and dried ($Na_2SO_4$). The solvent was evaporated and the product distilled: (+)-3-bromoheptane (2·5 g.), b. p. 64—70°/26 mm., $n_D^{25}$ 1·4481, $\alpha_{5893}^{21}$ +0·19° (*l* 0·5), was obtained which after redistillation had b. p. 65—67°/25 mm., $n_D^{25}$ 1·4482, $\alpha_{5893}^{21}$ + 0·18° (*l* 0·5) (Found: C, 47·1; H, 8·8; Br, 44·6. Calc. for $C_7H_{15}Br$: C, 46·9; H, 8·4; Br, 44·6%).

The silver salt (8·0 g.) of (−)-2-ethylhexanoic acid ($\alpha_{5893}^{27}$ −3·40°, *l* 0·5, 82% optically pure) was allowed to react similarly for 55 minutes at 0—10°; the filtrate was not heated. (−)-3-Bromoheptane (2·7 g.), b. p. 66—68°/26 mm., $\alpha_{5893}^{20}$ −0·07° (*l* 0·5), was obtained which after redistillation at the same b. p. had $n_D^{25}$ 1·4481, $\alpha_{5893}^{20}$ −0·06° (*l* 0·5) (Found: Br, 44·5%).

The silver salt (19·2 g.) of (+)-2-ethylhexanoic acid ($\alpha_{5893}^{20}$ + 3·74°, *l* 0·5, 92% optically pure) was allowed to react similarly for 2¾ hours at 10—15°; the filtrate was heated under reflux for 1 hour. (+)-3-Bromoheptane (4·6 g.), b. p. 65—68°/24 mm., $\alpha_{5893}^{25}$ +0·27° (*l* 2·0), was obtained which after redistillation had b. p. 63—65°/22 mm., $n_D^{25}$ 1·4482, $\alpha_{5893}^{23}$ +0·06° (*l* 0·5) (Found: Br, 44·6%).

To (±)-α-phenylpropionic acid (30 g.) was added N-sodium hydroxide (175 ml.); the solution

was titrated with further aqueous sodium hydroxide until just alkaline to phenolphthalein. The solution was warmed to 50° and to it was added, dropwise with stirring, a warm solution of silver nitrate (34 g.) in water (200 ml.); the precipitated silver (±)-α-phenylpropionate was washed with water, acetone, and ether and dried ($P_2O_5$) in a vacuum. It (50 g.) was finely ground and re-dried.

To a well-stirred suspension of silver (±)-α-phenylpropionate (10·5 g.) in carbon tetrachloride (80 ml.), boiling under reflux on a steam-bath, a solution of bromine (6·4 g.) in carbon tetrachloride (20 ml.) was added during 15 minutes. The reaction mixture was heated until the bromine colour had disappeared (1½ hours) and then for a further hour; it was allowed to cool, then filtered through a sintered-glass filter, and the filtrate treated as described above. On distillation an oil (4·0 g.) was obtained which boiled over the range 90—210°/17 mm. This product, combined with that from a similar experiment, was distilled; fractions of b. p. 90—182°/20 mm. and b. p. 182—194°/20 mm. were collected, both of which deposited crystals on cooling. These were filtered off and recrystallised from aqueous alcohol, bromine-free rhombs, m. p. 73°, of *1-phenylethyl α-phenylpropionate* being obtained (Found: C, 79·8; H, 6·9. $C_{17}H_{18}O_2$ requires C, 80·3; H, 7·1%). This ester (1·1 g.) was heated with potassium hydroxide (0·5 g.) in diethylene glycol (3 ml.) and yielded an alcohol, the phenylurethane of which had m. p. 88—89° alone and when mixed with an authentic specimen (m. p. 89°) of the phenylurethane of 1-phenylethyl alcohol. The acid obtained from this hydrolysis was converted into its silver salt, which was ignited [Found: Ag, (i) 42·6, (ii) 42·4. Calc. for $C_9H_9O_2Ag$: Ag, 42·0%]. Various procedures—addition of undiluted bromine to the hot silver salt suspension, similar addition at room temperature, addition of bromine in carbon tetrachloride at −15°, addition of the silver salt suspension to the bromine solution at 55°—followed by heating under reflux gave little product having b. p. approximating to that of 1-phenylethyl bromide (84°/10 mm.); widely-boiling fractions were obtained from which only 1-phenylethyl α-phenylpropionate was isolated.

Thanks are expressed to Dr. J. Kenyon, F.R.S., for his interest in this work, to the Department of Scientific and Industrial Research for a maintenance grant (to D. C. A.), and to Imperial Chemical Industries Limited for a grant.

BATTERSEA POLYTECHNIC, LONDON, S.W.11. [*Received, April 16th*, 1952.]

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## 613. *Some Trinuclear Cyanine Dyes. Part V.* Attempted Synthesis of Dyes Isomeric with Those of the Neocyanine Type.*

By FRANCES M. HAMER.

Four trinuclear trimethincyanines were synthesised by condensing a *meso*-methyltrimethincyanine with a heterocyclic aldehyde or with an anil. Two of them were also obtained by action of sodium ethoxide on a 2-methylbenzothiazolium salt and a 2-vinylbenzoxazolium salt having a heterocyclic substituent on the β-position of the vinyl group. Other *meso*-substituted trimethincyanines were prepared by the known method involving 2-substituted benzoxazolium salts. Absorption data are recorded; in three series a sequence unusual for cyanines is noted. A trinuclear trimethincyanine resisted quaternary salt formation.

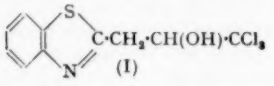
### A. HETEROCYCLIC ALDEHYDES

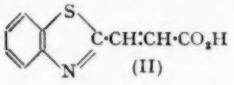
FOR synthesis of trinuclear cyanines, heterocyclic aldehydes were required. According to the original method, the addition compound of quinaldine and chloral had been hydrolysed to β-2-quinolylacrylic acid, which was oxidised by alkaline permanganate to 2-formylquinoline (von Miller and Spady, *Ber.*, 1885, **18**, 3402; 1886, **19**, 130; cf. Cooper and Cohen, *J.*, 1932, 723). Lepidine and chloral similarly gave an addition compound (von Miller and Spady, *loc. cit.*), which had been hydrolysed to β-4-quinolylacrylic acid (Koenigs and Müller, *Ber.*, 1904, **37**, 1337) and then similarly oxidised (Chem. Fabr. auf Aktien, B.P. 240,051/1925). Subsequently 4-formylquinoline was prepared by oxidising

* Part IV, *J.*, 1951, 294.

lepidine with selenium dioxide (Kwartler and Lindwall, *J. Amer. Chem. Soc.*, 1937, **59**, 524), and this method was applied to the preparation of 2-formylbenzothiazole (Borsche and Doeller, *Annalen*, 1938, **537**, 53). The yields of 4-formylquinoline obtained by direct oxidation could not be repeated, a better overall yield resulting by the three-stage method starting from chloral (Clemo and Hoggarth, *J.*, 1939, 1241); later it was explained that freshly prepared selenium dioxide is essential for satisfactory yields of 2- and 4-formylquinoline (Kaplan, *J. Amer. Chem. Soc.*, 1941, **63**, 2654).

In the present work, condensation of 2-methylbenzothiazole and 2-methylbenzoselenazole, respectively, with chloral, by the method used for quinaldine and lepidine (*loc. cit.*), yielded (I) and its selenium analogue. Although increased yields had been


(I)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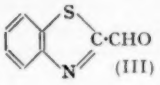

(III)

obtained by addition of pyridine in the preparation of analogous compounds from quinaldine (Alberts and Bachmann, *J. Amer. Chem. Soc.*, 1935, **57**, 1284) and from lepidine (Clemo and Hoggarth, *loc. cit.*), this addition proved disadvantageous with 2-methylbenzothiazole and -benzoselenazole. (I) was hydrolysed to (II), and the selenium analogue of (II) was also prepared. Similar preparations of (I) and (II) have recently been reported (Ettel, Weichet, and Chyba, *Coll. Czech. Chem. Comm.*, 1950, **15**, 528).

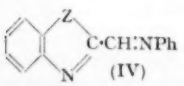
For oxidation of (II) to 2-formylbenzothiazole, the low temperature and high dilution prescribed for 4-formylquinoline (Clemo and Hoggarth, *loc. cit.*) were unsuccessful, and it had indeed been recorded that such conditions tend to the formation of dihydroxy-acids (Fittig, *Ber.*, 1888, **21**, 919). By use of hot concentrated aqueous solutions, 2-formylbenzothiazole (III) and 2-formylbenzoselenazole were prepared. Unfortunately the yields at each stage of the three-stage synthesis (52%, 61%, and 38% for the former, and 53%, 69%, and 48% for the latter) were below those (80%, 80%, and 58%) recorded in the synthesis of 4-formylquinoline (Clemo and Hoggarth, *loc. cit.*).

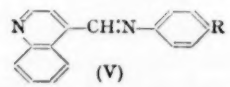
According to the equation :

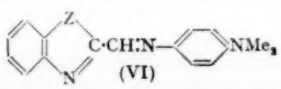
$$3R\cdot CH{:}CH\cdot CO_2K + 4KMnO_4 + 2H_2O = 3R\cdot CHO + 3H\cdot CO\cdot CO_2K + 4MnO_2 + 4KOH$$

three mols. of substituted acrylic acid should require four of potassium permanganate for oxidation to the aldehyde, but in fact about a 50% excess of permanganate had been used in making 2- (Cooper and Cohen, *loc. cit.*) and 4-formylquinoline (Chem. Fabr. auf Aktien, *loc. cit.*; Clemo and Hoggarth, *loc. cit.*). Comparative experiments now carried out on the oxidation of β-2-benzothiazolyl-, β-2-benzoselenazolyl-, and β-2-quinolyl-acrylic acid revealed that the maximum yield was not attained until for every 3 mols. of acid there had been added, not 4, but 7½—9 mols. of permanganate. This 100% excess is presumably used in oxidation of the accompanying glyoxylic acid, compared with which the heterocyclic aldehydes must be relatively stable. However, the latter were accompanied by small amounts of the corresponding 2-carboxylic acids, of which benzoselenazole-2-carboxylic acid was new.

There were prepared three new anils, namely (IV; Z = S, Se, or CH:CH), and the known (V; R = H) (Work, *J.*, 1942, 429), also (VI; Z = S or Se) and the known (VI;


(IV)

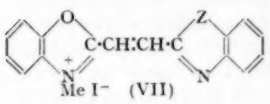

(V)


(VI)

Z = CH:CH) (Cooper and Cohen, *J.*, 1932, 723) and (V; R = $NMe_2$) (Ramsay, Baldwin, and Tipson, *J. Amer. Chem. Soc.*, 1947, **69**, 67). Although only two of these four *p*-dimethylaminoanils had been previously described, the quaternary salts of all of them were known, namely, the ethiodide of (VI; Z = S) (Hamer, *Phot. J.*, 1929, **69**, 409), the ethobromide of (VI; Z = Se) (Fisher and Hamer, *J.*, 1937, 907), and the ethiodides of (VI; Z = CH:CH) (Kaufmann and Vallette, *Ber.*, 1912, **45**, 1736) and of (V; R = $NMe_2$) (Hamer, *loc. cit.*); they had been characterised as photographic desensitisers (Hamer, *loc. cit.*;

*J.*, 1924, **125**, 1348; Bloch and Hamer, *Phot. J.*, 1930, **70**, 374; Fisher and Hamer, *loc. cit.*). Because of this desensitising action of the quaternary salts, it was surprising to find considerable photographic sensitisation produced by two of the bases, namely by (VI; Z = S or Se), whilst (VI; Z = CH:CH) also had some sensitising power; the fourth base (V; R = $NMe_2$) did not sensitise. Whereas their four quaternary salts gave purple solutions, with maxima (*locc. cit.*) varying bathochromically on passing along the series benzoselenazolium, benzothiazolium, 2-quinolinium, 4-quinolinium, solutions of the four bases were yellow, being readily decolorised by acid, and the maxima are in the sequence 2-quinoline, 4-quinoline, benzothiazole, benzoselenazole, as shown in the Table (p. 3201).

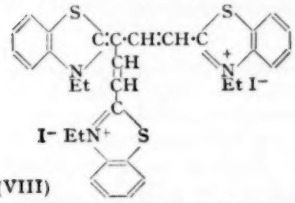
The compounds (VII; Z = S, Se, or CH:CH) were prepared by condensing the appropriate anils (IV; Z = S, Se, or CH:CH) with 2-methylbenzoxazole methiodide in ethanol. Yet attempts to condense either (III) or 2-formylquinoline with a 2-methylbenzoxazolium salt were unsuccessful: this was surprising in view of the reactivity of 2-formylquinoline with primary amines (Huang-Hsinmin and Mann, *J.*, 1949, 2903). The salts (VII) were unstable. No effect on a chlorobromide emulsion was detected. When they were arranged in order of increasing $\lambda_{max.}$, the sequence of nuclei linked to the 2-benzoxazolium nucleus was again 2-quinoline, benzothiazole, benzoselenazole, as recorded in the Table.



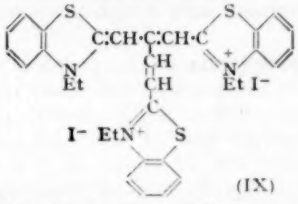
(VII)

### B. Trinuclear dyes

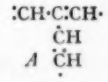
Trinuclear dyes of the neocyanine type have been proved to have an unbranched pentamethin chain, with the three nuclei symmetrically arranged, as in (VIII) (where only one of the resonating structures is formulated), whence they may be regarded as both γ-substituted pentamethin- and α-substituted trimethin-cyanines (Hamer, Rathbone, and Winton, *J.*, 1947, 1434; Kendall and Majer, *J.*, 1948, 690). According to a formula proposed by Brooker (B.P. 408,273/1931; cf. Brooker, Hamer, and Mees, *Phot. J.*, 1933, **57**, 258), they had been postulated, not as α-, but as β-substituted trimethincyanines, such as (IX). The synthesis of such unknown salts, which would be isomeric with neocyanines, now seemed of interest. Thus, 2-formylbenzothiazole might be used for synthesising (IX), and similarly, the 2- or the 4-aldehyde of the quinoline series, or the unknown 2-formylbenzoselenazole, seemed possible as starting points for other neocyanine isomers. These would all be characterised by having the chain (*A*) in one canonical form but it is to be noted that the number of structures contributing to the hybrid would be smaller than in the neocyanines themselves. The problem of preparing the required heterocyclic aldehydes has been dealt with in section *A*.



(VIII)

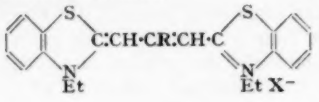


(IX)

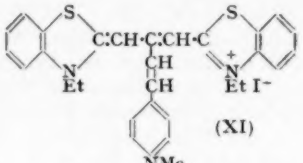


When considering these heterocyclic aldehydes for synthesis of neocyanines, two facts may be noted. On the one hand, the reactivity of 2-formylquinoline towards primary amines has been demonstrated (Huang-Hsinmin and Mann, *loc. cit.*). Quaternary salts of the heterocyclic aldehydes have not been described, though some such compounds were liberated by the action of acid on their *p*-dimethylaminoanils, and forthwith condensed with heterocyclic hydrazines (Fuchs and Grauaug, *Ber.*, 1928, **61**, 57). On the other hand, the reactivity of the β-methyl group of (X; R = Me, X = I) was discovered by Ogata, who condensed the ethiodide with benzaldehyde, *p*-dimethylaminobenzaldehyde (to give XI), and furfuraldehyde (*Bull. Chem. Soc. Japan*, 1936, **11**, 262). Although trinuclear cyanines, *e.g.*, (XII), had subsequently been made, by condensing a dye such as (X; R = Me) with a heterocyclic quaternary ammonium salt, having, *e.g.*, a reactive alkylthio-group

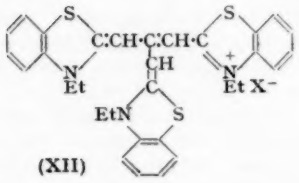
(Brooker, White, and Eastman Kodak Co., U.S.P. 2,282,115/1942), such dyes differ fundamentally from those of the neocyanine type in having only one anion, whereas the neocyanines have two.



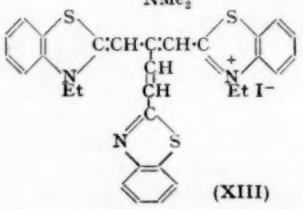
(X)



(XI)



(XII)

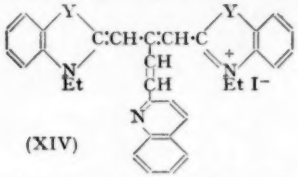


(XIII)

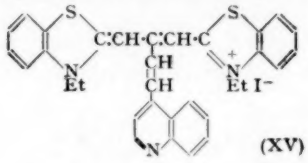
With the present aldehydes, unlike those used by Ogata (*loc. cit.*), condensation could not be effected with piperidine in alcohol, but the ethochloride (X; R = Me, X = Cl) condensed with (III) in pyridine. The product was isolated as iodide and most of the red impurity removed from the bluer dye (XIII) by means of aqueous pyridine. When the selenium analogue of (X; R = Me, X = Cl) was heated with 2-formyl-benzoselenazole or -benzothiazole in pyridine, the only product isolated was the dye iodide (Brooker, White, and Eastman Kodak Co., U.S.P. 2,058,406/1936) corresponding to the unchanged dye reactant. Similarly an attempt to condense (X; R = Me, X = Cl) with 2-formylquinoline in pyridine led to a red dye iodide and after recrystallisation there was a 52% recovery of (X; R = Me, X = I).

In spite of this negative result with 2-formylquinoline, the desired dye (XIV; Y = S) and its analogue (XIV; Y = Se) were prepared by condensing (X; R = Me, X = Cl) and its selenium analogue, respectively, not with the aldehyde, but with the anil (IV; Z = CH:CH). Similarly, from (X; R = Me, X = Cl), by use of (V; R = H), there was synthesised (XV). Yet an attempt to obtain an improved yield of (XIII) by condensing (X; R = Me, X = Cl) with the anil (IV; Z = S) instead of with (III), was unsuccessful and the only dye isolated was (X; R = Me, X = I).

Dr. L. G. S. Brooker had previously prepared both (XIII) and (XIV; Y = S) and failed to make quaternary salts of such compounds (personal communication).



(XIV)



(XV)

None of the four new trinuclear dyes showed appreciable sensitisation; all depressed the original sensitivity of the plate and this was particularly marked with (XIII) and (XV).

The absorption curves of methanolic solutions (stabilised with alkali) of the new trinuclear trimethincyanines were plotted; acidified solutions gave less intense absorption and were unstable. It has been recorded that the absorption maxima of the unsubstituted trimethincyanine (X; R = H), of its *meso*-methyl derivative (X; R = Me), and of its *meso*-phenyl derivative (X; R = Ph) lie, respectively, at 5575, 5430, and 5600 Å (Brooker and White, *J. Amer. Chem. Soc.*, 1935, **57**, 2480). When the *meso*-substitutent is the much larger β-2-quinolylvinyl group, the absorption maximum, 5600 Å, is the same as for the *meso*-phenyl compound; those of the analogues with a *meso*-β-4-quinolylvinyl group and a *meso*-β-2-benzothiazolylvinyl group are shown in the Table. In the corresponding

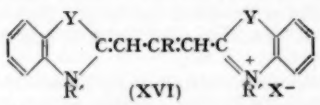
benzoselenazole series, the unsubstituted trimethincyanine has its absorption maximum at 5700 Å (Fisher and Hamer, *Proc. Roy. Soc.*, 1936, *A*, **154**, 703), the *meso*-methyl derivative at 5560 Å, and that with a *meso*-β-2-quinolylvinyl group at 5700 Å. From the close correspondence of the absorption maxima of (X; R = H), (X; R = Ph), and (XII), Bruylants, van Dormael, and Nys concluded that the *meso*-substitutent lies outside the plane of the conjugated resonating system (*Bull. Acad. Belg., Cl. Sci.*, 1948, **34**, 703; cf. van Dormael, *Bull. Soc. chim. Belg.*, 1949, **58**, 167). Since the absorption maxima of the four new *meso*-dyes lie so close to those of the corresponding unsubstituted trimethincyanines, it appears that here also the *meso*-substituent does not contribute to the charge resonance system.

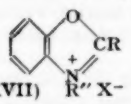
The trinuclear cyanines (XIV; Y = S), (XV), and (XIII), when tabulated according to the nature of the *meso*-substituent, fall into the same new sequence as the anils and the salts (VII). This is not a normal sequence for cyanines but in another series, where S or Se replaced CH:CH, the absorption maximum of styrene (Braude and Timmons, *J.*, 1950, 2000) was found to lie at a shorter wave-length than that of methyl phenyl sulphide or ethyl phenyl selenide (Bowden and Braude, *J.*, 1952, 1068).

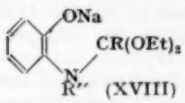
*Absorption maxima* (Å).

| Heterocyclic group | Trinuclear dyes | Anils (IV and V) | Benzoxazole derivatives (VII) |
|---|---|---|---|
| 2-Quinolyl | 5600 (XIV; Y = S) | 4180 | 3680 |
| 4-Quinolyl | 5610 (XV) | 4320 | — |
| 2-Benzothiazolyl | 5630 (XIV) | 4380 | 3850 |
| 2-Benzoselenazolyl | — | 4400 | 3880 |

A second synthesis of the β-substituted trimethincyanines, (XIII) and (XIV; Y = S), was based on that remarkable method, by which a β-substituted trimethincyanine (XVI; Y = S or Se) is prepared by breaking down, by means of sodium ethoxide, a benzoxazolium salt (XVII) having the desired substituent in the 2-position, and condensing the product with a 2-methylbenzo-thiazolium or -selenazolium salt (I.G. Farbenind. A.-G., B.P. 439,359; cf. B.P. 439,807/1933). The validity of the method was confirmed by preparing (X; R = Me, X = I) from 2-methylbenzoxazole methiodide, 2-methylbenzothiazole ethiodide, and sodium dissolved in alcohol, and identifying it with a standard sample. In certain particulars it was found desirable to deviate from the procedure of the patent: for instance, since the acetal formulated as intermediate (XVIII) (*loc. cit.*) requires three molecules of sodium ethoxide for its formation from (XVII), three atomic proportions


(XVI)


(XVII)


(XVIII)

of sodium were used, instead of two. The modified method was applied to the preparation of (X; R = $C_{11}H_{23}$ or $C_{15}H_{31}$, X = I), the latter of which is given in the patent, to the 5 : 5′- and 6 : 6′-dichloro-derivatives of (X; R = $C_{11}H_{23}$, X = I), to the 5 : 5′-, 6 : 6′-, and 7 : 7′-dichloro-derivatives of (X; R = $C_{15}H_{31}$, X = I), and to the 4 : 5 : 4′ : 5′-tetramethoxy-derivative of (X; R = $C_{15}H_{31}$, X = I). The 6 : 7 : 6′-7′-dibenzo-derivatives of (X; R = $C_{15}H_{31}$ or $C_{11}H_{23}$) were also prepared by this method, as was the selenium analogue of (X; R = $C_{15}H_{31}$) which is recorded in the patent. That the method is applicable to the introduction of substituents other than alkyl groups was exemplified in the patent and was now demonstrated by the preparation of (XI) from 2-methylbenzothiazole ethiodide and (XVII; R = CH:CH·$C_6H_4$·$NMe_2$, R″ = Et, X = $SO_3$·$C_6H_4$Me).

The compounds (XIII) and (XIV; Y = S) were prepared by this method depending on suitable salts (XVII), which were, specifically, (VII; Z = S or CH:CH).

Heating (VII; Z = S), 2-methylbenzthiazole ethiodide, and sodium ethoxide in alcohol gave a purple dye iodide. The same treatment with aqueous pyridine, which in the other method had been successful in removing the red component from (XIII), was applied here, and again successfully, although the proportion of desired dye to by-product was here far less. The present sample was identified with (XIII). From (VII; Z = CH:CH) and

2-methylbenzothiazole ethiodide, (XIV; Y = S) was similarly obtained, and similarly purified, but again in low yield.

The final stage of the synthesis was not accomplished, namely, the preparation of a diethiodide. These experiments were carried out with (XIV; Y = S) but it was recovered unchanged after being heated with ethyl iodide and decomposed when heated, at a higher temperature, with ethyl toluene-*p*-sulphonate.

## Experimental

Unless otherwise stated, samples for analysis were previously heated in a vacuum.

2-(3 : 3 : 3-*Trichloro*-2-*hydroxypropyl*)*benzothiazole* (I).—Chloral (38·94 g., 1 mol.) and 2-methylbenzothiazole (33·7 c.c., 1 mol.) were heated together on a steam-bath for 8 hours. The glassy product was crystallised from light petroleum (b. p. 60—80°; 3500 c.c.), giving a 52% yield. Recrystallisation from benzene (3·5 c.c. per g.) reduced the yield to 42%. The white crystals had m. p. 125° (Found: Cl, 35·8; S, 11·0. Calc. for $C_{10}H_8ONCl_3S$: Cl, 35·9; S, 10·8%). Ettel, Weichet, and Chyba reported a 31% yield and m. p. 126—127° (*Coll. Czech. Chem. Comm.*, 1950, **15**, 528).

2-(3 : 3 : 3-*Trichloro*-2-*hydroxypropyl*)*benzoselenazole*.—Chloral (38·38 g., 1 mol.) and 2-methylbenzoselenazole (1 mol.) were heated together as above. The powdered product was extracted with light petroleum (b. p. 60—80°; 3000 c.c.). The filtrate from the first crop of crystals was made up to the original volume for a second extraction and this was repeated twice more, giving a 53% yield of *propyl* derivative and a black residue. Recrystallisation from alcohol and water (3 c.c. and 1·5 c.c., respectively, per g.) reduced the yield to 47%. Nitrogen was determined by Dumas's method, and chlorine by the modified Carius method (Hamer, *Analyst*, 1933, **58**, 26) (Found: N, 4·2; Cl, 30·8. $C_{10}H_8ONCl_3Se$ requires N, 4·1; Cl, 30·95%). The buff crystals had m. p. 122° with previous softening.

2-(3 : 3 : 3-*Trichloro*-2-*hydroxypropyl*)*quinoline*.—Instead of using the method of von Miller and Spady (*Ber.*, 1885, **18**, 3402; 1886, **19**, 130), who do not quote a yield, that used by Clemo and Hoggarth for the 4-quinoline isomer (*J.*, 1939, 1241) was modified. Quinaldine (39 c.c., 1 mol.), chloral (1 mol.), and anhydrous pyridine (100 c.c.) were heated together on a water-bath for 3 hours and the solution was poured into water (500 c.c.). The product was recrystallised from ethanol (300 c.c.) and well washed; it (72%) had m. p. 146—147°, whereas von Miller and Spady recorded 144—145°.

4-(3 : 3 : 3-*Trichloro*-2-*hydroxypropyl*)*quinoline*.—Freshly distilled lepidine (41 c.c., 1 mol.), freshly prepared chloral (1 mol.), and anhydrous pyridine (100 c.c.) were heated together on a boiling water-bath for 5 hours and the dark liquid was poured into water (500 c.c.). The resultant solid, recrystallised from ethanol (450 c.c.), gave a 62% yield of almost colourless crystals, m. p. 176—177°. When heating was for only 2 hours, the yield was 43%. Clemo and Hoggarth (*loc. cit.*) prescribed 2 hours' heating at 85—90° and recorded an 80% yield and m. p. 178°.

β-2-*Benzothiazolylacrylic Acid* (II).—2-(3 : 3 : 3-Trichloro-2-hydroxypropyl)benzothiazole (22·0 g., 1 mol.) was added, during 5 minutes, to an initially boiling solution of potassium hydroxide (18·3 g., 4·4 mols.) in ethanol (375 c.c.), with vigorous mixing. The heat of reaction kept up ebullition. After the addition, the mixture was boiled and stirred for 10 minutes. After cooling, the potassium salts were filtered off, washed thrice with alcohol, and dried. They were dissolved in boiling water (210 c.c.) and the acid was precipitated with acetic acid. After cooling, it was filtered off and well washed with water (yield, 61%) (Found: C, 58·5; H, 3·55. Calc. for $C_{10}H_7O_2NS$: C, 58·5; H, 3·45%). The almost colourless solid had m. p. 215° (decomp.). Ettel, Weichet, and Chyba (*loc. cit.*) gave m. p. 218—219° but not a yield.

Alternatively, 2-(3 : 3 : 3-trichloro-2-hydroxypropyl)benzothiazole (14·8 g., 1 mol.) was added, in one lot, to a hot solution of sodium (4 atoms) in ethanol (250 c.c.) and, when the reaction had abated, boiling and stirring were applied for 10 minutes. The sodium salts were filtered off hot, washed with ethanol, and dissolved in hot water (200 c.c.), and the acid was precipitated by acetic acid in 55% yield. The filtrates from three such preparations were heated, acidified with acetic acid, and added to four volumes of hot water; the acid obtained on cooling was purified through its potassium salt, bringing the yield to 71%.

β-2-*Benzoselenazolylacrylic Acid*.—2-(3 : 3 : 3-Trichloro-2-hydroxypropyl)benzoselenazole (17·2 g., 1 mol.), as in the second method above, gave the *acid* in 48% yield. A sample was purified through its potassium salt, the yield of washed acid being 43% [Found: N (Dumas), N, 5·35. $C_{10}H_7O_2NSe$ requires N, 5·55%]. The almost colourless crystals had m. p. 260°

(decomp.), with previous shrinking. The original filtrates from three such preparations were acidified with acetic acid and added to four volumes of hot water. The product was boiled with potassium carbonate (2 mols. per mol.) in water (300 c.c.), with addition of charcoal (1 g.), and the solution filtered hot. Precipitation with acetic acid gave a 21% yield of the acid, bringing the total to 69%.

β-2-*Quinolylacrylic Acid.*—After 2-(3 : 3 : 3-trichloro-2-hydroxypropyl)quinoline had been boiled with potassium carbonate solution for 3—4 hours, as described by von Miller and Spady (*loc. cit.*), who do not give a yield, addition of excess of hydrochloric acid was necessary, rather than the careful neutralisation they recommend, the yield being 43%. The following procedure was better.

2-(3 : 3 : 3-Trichloro-2-hydroxypropyl)quinoline (55 g., 1 mol.) was gradually added to an initially boiling solution of potassium hydroxide (5·2 mols.) in ethanol (255 c.c.), during 25 minutes, with vigorous mixing. Boiling and stirring were applied for a further 40 minutes. After cooling, the potassium salts were filtered off, washed thrice with ethanol, and dried. They were dissolved in boiling water (165 c.c.), and the acid was precipitated hot with acetic acid. After cooling, it was filtered off and washed with water (yield, 58%). It was nearly colourless and had m. p. 192—194° (decomp.), with previous darkening, whereas the recorded m. p. (*loc. cit.*) was 190—195°. On recrystallisation from methanol (20 c.c. per g.), the yield dropped to 38% but the m. p. was not changed (Found: C, 72·5; H, 4·75. Calc. for $C_{12}H_9O_2N$: C, 72·35; H, 4·55%).

β-4-*Quinolylacrylic Acid.*—4-(3 : 3 : 3-Trichloro-2-hydroxypropyl)quinoline (55·5 g.) was similarly hydrolysed. Clemo and Hoggarth, on a larger scale (65 g.), took 2 hours for the addition, which was followed by an hour's further heating (*J.*, 1939, 1241). Although they found the solid, which they filtered off on cooling, to be only potassium chloride, in the present work it comprised also the bulk of the desired acid, in the form of its potassium salt. Instead, therefore, of their procedure of filtering at this stage, the reaction mixture was added to hot water (1300 c.c.), and the acid precipitated with acetic acid. After cooling, it was filtered off and well washed with water [yield, 76%; cf. Clemo and Hoggarth, 80% of purified acid, m. p. 270° (slight decomp.)]. The m. p. was 260° (decomp.) and was unaltered by recrystallisation from acetic acid (20 c.c. per g.), whereby the yield of colourless crystals dropped to 59% (Found: C, 72·1; H, 4·6. Calc. for $C_{12}H_9O_2N$: C, 72·35; H, 4·55%).

2-*Formylbenzothiazole* (III).—A solution of β-2-benzothiazolylacrylic acid (2·05 g., 3 mols.) and anhydrous potassium carbonate (6 mols.) in water (10 c.c.), together with chloroform (100 c.c.), was heated on a steam-bath, with vigorous stirring. A solution of potassium permanganate (3·96 g., 7½ mols.) in water (75 c.c.) was dropped in during 8½ minutes, and boiling and stirring were continued for 2 minutes more. The manganese dioxide was filtered off hot and the layers were separated. The precipitate was washed with the hot aqueous layer and with chloroform (30 c.c.), and this was repeated. The chloroform extracts were dried ($Na_2SO_4$) and evaporated in a vacuum. The residual aldehyde (53% yield), recrystallised from light petroleum (b. p. 60—80°; 10 c.c. per g.), in 38% yield, had m. p. 73°, with previous softening, whereas Borsche and Doeller give m. p. 65° (*Annalen*, 1938, **537**, 53) (Found, after drying in a vacuum-desiccator: S, 19·65. Calc. for $C_8H_5ONS$: S, 19·65%). Similar experiments with 5, 6, 6½, 7, and 8 mols. of permanganate (in 50 c.c., 60—80 c.c. of water) gave crude yields of, respectively, 30, 49, 48, 51, and 47%, although the calculated amount is only 4 mols.

On evaporation of the original aqueous filtrate to crystallisation, potassium benzothiazole-2-carboxylate (average 0·55 g.) was obtained (cf. Hofmann, *Ber.*, 1887, **20**, 2251). After two recrystallisations from water (10 c.c. per g.; about 50% loss each time), the pale buff plates, which remained unmelted at 270°, were analysed (Found, after drying in a vacuum-desiccator: C, 44·7; H, 1·95; N, 6·45; S, 15·0. Calc. for $C_8H_4O_2NSK$: C, 44·2; H, 1·85; N, 6·45; S, 14·75%. Determinations of C and H were by Drs. Weiler and Strauss).

Benzothiazole-2-carboxylic acid has been described and its ready breakdown to benzothiazole noted (Hofmann, *loc. cit.*; Reissert, *Ber.*, 1904, **37**, 3708).

2-*Formylbenzoselenazole.*—A solution of β-2-benzoselenazolylacrylic acid (2·52 g., 3 mols.) and anhydrous potassium carbonate (6 mols.) in hot water, together with chloroform, was treated with aqueous potassium permanganate (7½ mols.) as in the preceding preparation. The *aldehyde* from six such runs (48% yield) was recrystallised from light petroleum (b. p. 60—80°; 10 c.c. per g.), and obtained in 41% yield [Found, after drying in a vacuum-desiccator: N (Dumas), 6·95. $C_8H_5ONSe$ requires N, 6·65%]. The buff crystals had m. p. 72°. Similar experiments with 4, 5, 6, 6½, 7, 8, 9, and 10 mols. of permanganate (in 40—100 c.c. of water) gave crude yields of, respectively, 22, 28, 34, 40, 42, 46, 49, and 35%.

*Potassium Benzoselenazole-2-carboxylate.*—The original aqueous layer from six experiments as above with 7½ mols. of permanganate was concentrated until crystallisation began. The washed *potassium* salt (3·36 g.) was twice recrystallised from water (3 c.c. per g.; 1·82 g. obtained) [Found: N (Dumas), 5·45. $C_8H_4O_2NKSe$ requires N, 5·3%]. The pale beige crystals remained unmelted at 270°.

*Benzoselenazole-2-carboxylic Acid.*—The twice recrystallised potassium salt (2 g.) was added to hot water (40 c.c.), the boiling solution treated with excess of hydrochloric acid, and the precipitated *acid* filtered off and washed (1·27 g.) (Found, after drying in a vacuum-desiccator: C, 42·85; H, 2·4; N, 6·45. $C_8H_5O_2NSe$ requires C, 42·4; H, 2·25; N, 6·2%. C and H determinations were by Drs. Weiler and Strauss). The cream-coloured product had m. p. about 120° (decomp.), depending on the rate of heating.

2-*Formylquinoline.*—A solution of β-2-quinolylacrylic acid (1·99 g., 3 mols.) and anhydrous potassium carbonate (6 mols.) in hot water, together with chloroform, was treated with aqueous potassium permanganate (7½ mols.) as in preceding instances. The aldehyde (80% yield), recrystallised from light petroleum (b. p. 60—80°; 6 c.c. per g.) (54% yield), was buff and had m. p. 69°, whereas 70—71° is recorded (von Miller and Spady, *Ber.*, 1885, **18**, 3402; 1886, **19**, 130). This preparation was satisfactorily carried out on a scale thrice as great. Similar small-scale experiments with 6, 8, 8½, and 10 mols. of permanganate gave crude yields of, respectively, 71, 80, 76, and 71%. On concentration of the original aqueous layer, less potassium salt crystallised than with the corresponding benzothiazole and benzoselenazole compounds.

4-*Formylquinoline.*—In a similar experiment with β-4-quinolylacrylic acid (4·98 g.), addition of permanganate took ½ hour and subsequent boiling and stirring were for 2 minutes. After recrystallisation of the product (2·25 g.) from light petroleum, b. p. 40—60°, the yield dropped to 33% (1·29 g.), m. p. 52°. The colourless crystals were strongly sternutatory. Cooling with ice during the oxidation did not give a better yield and, as the manganese dioxide was not coagulated, filtration was excessively slow. It did not seem practicable to apply to an aqueous solution prolonged cooling with a freezing mixture, as recommended by Clemo and Hoggarth, who, however, recorded a 58% yield (*J.*, 1939, 1241).

2-*Anilomethylbenzothiazole* (IV; Z = S).—Mixing 2-formylbenzthiazole (0·82 g., 1 mol.) and aniline (1·1 mols.) caused evolution of heat. After 30 minutes at 115—120°, the melt solidified on cooling. The product was dissolved in hot methanol (20 c.c.). The nearly colourless *base* (62%), m. p. 102°, crystallised (Found: C, 70·25; H, 4·25; N, 12·05. $C_{14}H_{10}N_2S$ requires C, 70·5; H, 4·25; N, 11·75%). For microanalyses of this and the following eleven compounds, I am indebted to Mr. F. H. Oliver of the Imperial College.

2-*Anilomethylbenzoselenazole* (IV; Z = Se).—A mixture of 2-formylbenzoselenazole (1·05 g., 1 mol.) and aniline (1·1 mols.) was heated at 98—101° for 5 minutes and the product dissolved in hot methanol (35 c.c.). The buff *anil* had m. p. 119° (Found: C, 58·7; H, 3·6; N, 9·85. $C_{14}H_{10}N_2Se$ requires C, 58·9; H, 3·55; N, 9·8%).

*Aniloquinaldine* (IV; Z = CH:CH).—2-Formylquinoline (0·78 g., 1 mol.) and aniline (1·1 mols.) were heated at 98—104° for 5 minutes. Each reaction mixture, of ten such, was treated with hot ethanol (2 c.c.), and the tubes were further washed with hot ethanol (½ c.c × 10). From the united filtrates the base crystallised in 67% yield. The filtrate and washings were treated hot with an equal volume of water, and a second crop (2·03 g.) crystallised on cooling. Its recrystallisation from light petroleum gave a 16% yield, making the total 83%. The *base* had m. p. 73—74°, with previous softening, and the m. p. of a sample was unchanged by recrystallisation from light petroleum (b. p. 60—80°; 8 c.c. per g.), whilst the yield dropped to 51% (Found: C, 82·35; H, 5·15; N, 12·0. $C_{16}H_{12}N_2$ requires C, 82·7; H, 5·2; N, 12·05%).

*Anilolepidine* (V; R = H).—A mixture of 4-formylquinoline (0·78 g., 1 mol.) and aniline (1·1 mols.) was heated according to Work's method (*J.*, 1942, 429). After being dried overnight in a vacuum-desiccator, the product was dissolved in warm ether (10 c.c.) and the solution treated with light petroleum (b. p. 40—60°; 10 c.c.). The crystals, m. p. 84°, were obtained in 76% yield; Work obtained a 79% yield (m. p. 85°).

2-p-*Dimethylaminoanilomethylbenzothiazole* (VI; Z = S).—On mixing of 2-formylbenzothiazole (0·41 g., 1 mol.) and *NN*-dimethyl-*p*-phenylenediamine (1 mol.), a yellow colour developed. After 5 minutes' heating at 115—120°, the solid was recrystallised from methanol (110c.c.), giving orange crystals (71%), m. p. 176° with previous shrinking, of *anil* (Found: C, 68·4; H, 5·55. $C_{16}H_{15}N_3S$ requires C, 68·3; H, 5·4%). The orange colour of an alcoholic solution was unchanged by addition of acetic acid but discharged by sulphuric acid and, after a short time, could not be restored by ammonia; with neither acid was there a purple colour resembling that of the ethiodide. A methanolic solution containing ammonia had an absorption maximum

at 4380 Å. This and the absorption curves of the following six compounds were obtained with a Hilger spectrograph. The dye strongly sensitised an iodobromide emulsion, sensitivity extending to 6100 Å, with the maximum at 5300 Å.

p-*Dimethylaminoanilomethylbenzoselenazole* (VI; Z = Se).—A mixture of 2-formylbenzoselenazole (0·52 g., 1 mol.) and *NN*-dimethyl-*p*-phenylenediamine (1 mol.) was similarly heated; recrystallisation of the product from benzene (7 c.c.), gave a 64% yield of the orange *base*, m. p. 186° (Found: C, 58·7; H, 4·3. $C_{16}H_{15}N_3Se$ requires C, 58·5; H, 4·6%). A methanolic solution containing ammonia had an absorption maximum at 4400 Å. Like the preceding base, this also was a strong sensitiser, with the same range and maximum.

p-*Dimethylaminoaniloquinaldine* (VI; Z = CH:CH).—Prepared as were the preceding two bases but from 2-formylquinoline (0·39 g.), the product separated from methanol (25 c.c.) as yellow crystals (64%), m. p. 145° (Found: C, 78·35; H, 6·25. Calc. for $C_{18}H_{17}N_3$: C, 78·5; H, 6·25%). Cooper and Cohen, who do not give a yield, recorded m. p. 148—150° (*J.*, 1932, 723). The yellow alcoholic solution on treatment with drops of dilute sulphuric acid gave an evanescent purple colour but excess of acid caused decolorisation. A methanolic solution containing ammonia had an absorption maximum at 4180 Å. The compound did not sensitise an iodobromide emulsion but somewhat decreased the original sensitivity; it sensitised a chlorobromide emulsion weakly, from 5200 to 5700 Å.

p-*Dimethylaminoanilolepidine* (V; R = $NMe_2$).—Similarly prepared from 4-formylquinoline (0·39 g.), the product crystallised from methanol (30 c.c.) in glistening red crystals (72%), m. p. 155° (Found: C, 78·9; H, 6·2%). A 94% yield and m. p. 156·2—157·2°, have been recorded (Ramsey, Baldwin, and Tipson, *J. Amer. Chem. Soc.*, 1947, **69**, 67). This base, in a methanolic solution containing ammonia, had a maximum at 4320 Å. It did not sensitise a chlorobromide emulsion.

2-(2-2′-*Benzothiazolylvinyl*)*benzoxazole Methiodide* (VII; Z = S).—A mixture of 2-methylbenzoxazole methiodide (0·14 g., 1 mol.), 2-anilomethylbenzothiazole (1 mol.), and acetic anhydride (0·1 c.c.) was heated at 83—88° for 15 minutes; crystallisation began at the end of 3 minutes. To remove acetanilide, the product was extracted with hot water (3 × 5 c.c.), and the residue heated with acetone (0·5 c.c.). After cooling, the *iodide* was filtered off and well washed with acetone (yield, 14%) (Found: C, 48·5; H, 3·3. $C_{17}H_{13}ON_2IS$ requires C, 48·55; H, 3·15%). The absorption maximum of a methanolic solution was at 3850 Å. The crystals were bright orange and had m. p. 202° (decomp.). Recrystallisation from acetic anhydride (100 c.c. per g.) did not raise the m. p., whilst reducing the yield to 3%, and attempted recrystallisation from methanol resulted in decomposition.

A better method was to heat 2-methylbenzoxazole methiodide (1·38 g., 1 mol.), 2-anilomethylbenzothiazole (1 mol.), and ethanol (11 c.c.) at 78—83° for 6 minutes: after 3 minutes, the solids had dissolved and the product began to crystallise. After cooling, the crystals were filtered off and washed thrice with alcohol, thrice with water, then again with alcohol, and were isolated in 29% yield. Addition of anhydrous sodium acetate to the reactants led to a negative result.

Attempted preparations from 2-formylbenzothiazole were unsuccessful.

2-(2-2′-*Benzoselenazolylvinyl*)*benzoxazole Methiodide* (VII; Z = Se).—A mixture of 2-methyl benzoxazole methiodide (1·38 g., 1 mol.), 2-anilomethylbenzoselenazole (1 mol.), and ethanol (11 c.c.) was heated and stirred by hand at 78—83° for 6 minutes. After cooling, the crystals were filtered off and washed, leaving a 34% yield. The absorption maximum of a methanolic solution was at 3880 Å. Recrystallisation of the bright reddish-orange *salt* from acetic anhydride (130 c.c. per g.) caused the yield to drop to 3%, whilst the m. p. rose from 195° (decomp.) to 197° (decomp.) (Found: C, 43·7; H, 3·1. $C_{17}H_{13}ON_2ISe$ requires C, 43·65; H, 2·8%). None of the desired product was obtained when sodium acetate was included with the reactants.

The preparation was also successful from 2-methylbenzoxazole methiodide and 2-anilomethylbenzoselenazole in acetic anhydride, but unsuccessful when 2-formylbenzoselenazole was used in this way instead of its anil.

2-(2-2′-*Quinolylvinyl*)*benzoxazole Methiodide* (VII; Z = CH:CH).—2-Methylbenzoxazole methiodide (1·38 g., 1 mol.) and aniloquinaldine (1 mol.) in ethanol (11 c.c.) were stirred together and heated at 79—83° for 1 minute. After cooling, the deep yellow crystals were filtered off and washed (yield, 45%). Recrystallisation from acetic anhydride (145 c.c. per g.) reduced the yield to 8% but the m. p., 193° (decomp.), was unaltered. The absorption maximum of a methanolic solution was at 3680 Å, with secondary maxima at 2810 and 2920 Å. The absorption band was no longer shown by a solution which had been left overnight (Found, for the washed

specimen : C, 54·75; H, 3·8; N, 7·25. $C_{19}H_{15}ON_2I$ requires C, 55·05; H, 3·65; N, 6·75%). Attempted recrystallisation from ethanol (110 c.c. per g.) resulted in a total loss. An attempted preparation by heating together 2-methylbenzoxazole methiodide, 2-formylquinoline, sodium acetate, and ethanol for 45 minutes (Kendall, B.P. 495,197/1937, Ex. 16) was unsuccessful, as would be expected from the instability of the salt.

*Bis*-2-(3-*ethylbenzthiazole*)-β-*methyltrimethincyanine Iodide* (X; R = Me, X = I).—To a mixture of 2-methylbenzoxazole methiodide (1·38 g., 1 mol.) and 2-methylbenzothiazole ethiodide (2 mols.) in boiling ethanol (7·5 c.c.) was added a solution of sodium (3 atoms) in hot ethanol (10 c.c.), and boiling was continued for only 2 minutes, which was much less than the times in B.P. 439,359. The preliminary heating, there described, of the benzoxazolium salt with sodium ethoxide, before addition of the benzothiazolium salt, proved harmful and was omitted. After cooling, the dye was filtered off, washed and extracted with hot methanol (10 c.c.), and the residue (41%) was recrystallised from methanol (200 c.c. per g.), giving a 34% yield (Found : I, 24·95. Calc. for $C_{23}H_{23}N_2IS_2$ : I, 25·05%). The dye had the same absorption and sensitising curves, m. p. and mixed m. p., as had a specimen prepared by use of ethyl orthoacetate (Hamer, *J.*, 1928, 3160).

2-*Undecylbenzoxazole and its Methomethylsulphate*.—This base had been prepared by use of dodecanoamide (Bywater, Coleman, Kamm, and Merritt, *J. Amer. Chem. Soc.*, 1945, **67**, 905). In the present work dodecanoic acid (18·3 g., 1 mol.) and *o*-aminophenol (1 mol.) were heated together at 200—220° for 12 hours. To remove unchanged acid, the mixture was ground with 4% sodium hydroxide solution (3 × 200 c.c.). In the presence of another such quantity, the base was then extracted with benzene. After drying of the extract and removal of the solvent, vacuum-distillation, after removal of a forerun, gave a 62% yield of material, b. p. 225—245°/35—50 mm., which solidified. To convert it into its methomethylsulphate, the base (0·68 g., 1 mol.) was heated at 100—110° for 2 hours with neutral methyl sulphate (1·25 mols.).

*Bis*-2-(3-*ethylbenzthiazole*)-β-*undecyltrimethincyanine Iodide* (X; R = $C_{11}H_{23}$, X = I).—To the above gummy salt were added 2-methylbenzothiazole ethiodide (2 mols.) and ethanol (5 c.c.), and the mixture was boiled. After addition of a hot solution of sodium (3 atoms) in ethanol (5 c.c.), boiling was continued for 2 minutes. The yield of washed *product*, after crystallisation from ethanol (15 c.c.), amounted to 53%. To obtain an analytically pure specimen, three recrystallisations were used, the first and third from ethanol (25 c.c. per g.) and the second from methanol (7 c.c. per g.) (40% yield) (Found : I, 19·95. $C_{32}H_{43}N_2IS_2$ requires I, 19·65%). The green crystals decomposed at about 120°. A methanolic solution had its absorption maximum at 5500 Å. The sensitising maximum was at 5900 Å, with a secondary one at 5200 Å, and sensitisation extended to 6300 Å; the sensitising action of this and the following dyes was in gelatino-bromide emulsions.

2-*Pentadecylbenzoxazole and its Methomethylsulphate*.—The base was originally prepared by heating the reactants together under reduced pressure (Fierz-David and Kuster, *Helv. Chim. Acta*, 1939, **22**, 82) but the vacuum appears unnecessary (I.G. Farbenind. A.-G., B.P. 439,359/1933). In the present work *o*-aminophenol (10 g., 1 mol.) and palmitic acid (1 mol.) were heated together at 205—220° for 12 hours, in a flask with a short air-condenser, which allowed water vapour to escape. The molten product was poured out and the brown cake ground thrice with sodium hydroxide solution, extracted with benzene, dried, and finally distilled, resulting in material (70%) of b. p. 275°/30—35 mm., m. p. 43·5°, compared with m. p. 45·5° recorded by Fierz-David and Kuster (*loc. cit.*). On 6 hours' heating, the yield was 63% and on 3 hours' heating was only 39%. To convert the base into its *methomethylsulphate*, it (4 g.; 1 mol.) was heated for 2 hours at 100—110° with neutral methyl sulphate (1·2 mols.). The syrupy product was inoculated whilst hot and stirred during cooling. After grinding with absolute ether (40 c.c.), there resulted an 83% yield of almost pure salt (Found : S, 7·4%), which was used for dye preparations. For analysis, the product was twice ground with dry acetone (9 c.c., 25 c.c.), and washed with it (25 c.c.), giving a 48% yield (Found, after drying in a vacuum-desiccator : S, 7·35. $C_{24}H_{41}O_5NS$ requires S, 7·05%). The white salt, which no longer appeared hygroscopic, had an indefinite m. p., softening from about 85° but only becoming clear at about 142°.

*Bis*-2-(3-*ethylbenzthiazole*)-β-*pentadecyltrimethincyanine Iodide* (X; R = $C_{15}H_{31}$, X = I).—A mixture of 2-pentadecylbenzoxazole methomethylsulphate (1·14 g., 1 mol.) and 2-methylbenzothiazole ethiodide (2 mols.) in boiling ethanol (5 c.c.) was treated with sodium (3 atoms) in ethanol (5 c.c.) and boiled for 2 minutes. The washed product was dissolved in hot alcohol (10 c.c.) and treated with a hot solution of its own weight of potassium iodide, dissolved in water (10 c.c.). The tar hardened on cooling and was ground with water (46% yield); it crystallised from ethyl acetate (30 c.c.), giving a 32% yield. After recrystallisation from ethyl acetate

(95 c.c. per g.), the yield was 26% (Found, after drying in a vacuum-desiccator: I, 17·9. $C_{36}H_{51}N_2I$ requires I, 18·05%). The green *iodide* began to shrink at 80—90°, melted at 124° to a viscous liquid, which became more mobile about 185°, and decomposed at about 210°. The absorption maximum of a methanolic solution was at 5450 Å, and the sensitising maximum at 5800 Å. This is example 5 of B.P. 439,359, but none of its properties is described there.

*Bis*-2-(5-*chloro*-3-*ethylbenzothiazole*)-β-*undecyltrimethincyanine Iodide*.—2-Undecylbenzoxazole (2·73 g.) and methyl sulphate were heated together at 100—120° for 2 hours. To the crude salt were added 5-chloro-2-methylbenzothiazole ethiodide and ethanol (20 c.c.), and the mixture was boiled. Undissolved solids were present until a solution of sodium in alcohol was added. Boiling and stirring were for 4 minutes. Crystallisation of the washed *product* from ethanol (140 c.c.) gave a 25% yield, and recrystallisation from methanol (70 c.c. per g.) a 19% yield. The crystals consisted of a less stable red and a more stable green form. After another recrystallisation from methanol, the yield was 16% (Found: 2Cl + I, 27·9. $C_{32}H_{41}N_2Cl_2IS_2$ requires 2Cl + I, 27·65%). Shrinking and darkening, with loss of crystalline form, took place at about 95°. Melting began at about 185° and frothing about 205°, but these temperatures depended on the rate of heating. A methanolic solution had its absorption maximum at 5550 Å. Sensitising was weak, with the maximum at 5995 Å.

*Bis*-2-(6-*chloro*-3-*ethylbenzothiazole*)-β-*undecyltrimethincyanine Iodide*.—In a preparation carried out similarly but with 6-chloro-2-methylbenzothiazole ethiodide, the washed product was boiled with, and recrystallised from, ethanol (30 c.c., 940 c.c.), giving a 25% yield. After a second recrystallisation from methanol (150 c.c. per g.), the yield was 21% (Found: 2Cl + I, 27·85. $C_{32}H_{41}N_2Cl_2IS_2$ requires 2Cl + I, 27·65%). The bluish-green *iodide* darkened from about 170°; the m. p. was at about 202° (decomp.) but depended on the rate of heating. A methanolic solution had the same absorption maximum as the preceding dye. Sensitivity extended to 6400 Å, with the maximum at 6000 Å.

*Bis*-2-(5-*chloro*-3-*ethylbenzothiazole*)-β-*pentadecyltrimethincyanine Iodide*.—To a hot mixture of 2-pentadecylbenzoxazole methomethylsulphate (2·28 g.) and 5-chloro-2-methylbenzothiazole ethiodide in ethanol (10 c.c.) was added sodium dissolved in ethanol, and boiling was continued for 2 minutes. After being washed, the product was recrystallised from ethanol (30 c.c.), leaving a residue, and giving a 24% yield. After a second recrystallisation from ethanol (20 c.c. per g.), the yield of *salt* was 20% (Found, after drying in a vacuum at 50—60°: 2Cl + I, 24·85. $C_{36}H_{49}N_2Cl_2IS_2,\frac{1}{2}C_2H_5OH$ requires 2Cl + I, 24·9%. Drying in a vacuum-desiccator caused less loss of weight, whilst at 60—80° melting occurred). The green crystals began to shrink at about 88° but with no definite m. p.; violent decomp. occurred at about 200°. The absorption maximum of a methanolic solution was at 5500 Å. The dye sensitised weakly from 5200 to 6100 Å, with the maximum at 5800 Å.

*Bis*-2-(6-*chloro*-3-*ethylbenzothiazole*)-β-*pentadecyltrimethincyanine Iodide*.—In a similar preparation but with 6-chloro-2-methylbenzothiazole ethiodide, the *dye* crystallised from the purple solution. After washing and two recrystallisations from methanol (250 c.c. per g.) the yield was 17% (Found: 2Cl + I, 25·6. $C_{36}H_{49}N_2Cl_2IS_2$ requires 2Cl + I, 25·65%). The dark green crystals showed sudden shrinking at about 177° and violent decomp. at about 213°. The absorption maximum of a methanolic solution was at 5550 Å. Sensitisation was weak, with the maximum at 5900 Å.

*Bis*-2-(7-*chloro*-3-*ethylbenzothiazole*)-β-*pentadecyltrimethincyanine Iodide*.—In a similar preparation with 7-chloro-2-methylbenzothiazole ethiodide, the crude washed dye was recrystallised from ethanol (10 c.c.) and obtained in 20% yield. This was boiled with ethanol (11 c.c. per g.), and the product (14%) from the extract was again recrystallised from ethanol (30 c.c. per g.), giving an 8% yield (Found: 2Cl + I, 25·7. $C_{36}H_{49}N_2Cl_2IS_2$ requires 2Cl + I, 25·65%). The *dye* crystallised in a more soluble bronze and green and less soluble blue form. It had no definite m. p. Shrinking occurred from 170° with gradual melting (decomp.) between 180° and 192°. A methanolic solution had its absorption maximum at 5500 Å. There was poor sensitising from 5100 to 6100 Å, with the maximum at 5800 Å.

*Bis*-2-(3-*ethyl*-5: 6-*dimethoxybenzothiazole*)-β-*pentadecyltrimethincyanine Iodide*.—In a similar preparation with 5: 6-dimethoxy-2-methylbenzothiazole ethiodide, the hot reaction mixture was added to ten times its volume of water, containing potassium iodide. The washed *dye* was boiled with ethanol (60 c.c.), from which it crystallised in 24% yield. After two further crystallisations from ethanol (15 c.c. per g.), the yield was 13% (Found: I, 15·7. $C_{40}H_{59}O_4N_2IS_2$ requires I, 15·45%). The dark green crystals showed some decomp. by 120° and were molten by 140° but without a definite m. p.; on more rapid heating, melting and decomposition to a green tar occurred at about 130°. A methanolic solution had its absorption

maximum at 5800 Å. Moderate sensitisation extended to 6800 Å with maxima at 5400 and 6200 Å.

*Bis*-2-[3-*ethylnaphtho*(2′ : 1′-4 : 5)*thiazole*]-β-*pentadecyltrimethincyanine Iodide*.—In a preparation similar but with 2-methylnaphtho(2′ : 1′-4 : 5)thiazole ethiodide, the washed *product* was recrystallised from methanol (100 c.c.), giving a 39% yield. After a second such recrystallisation (65 c.c. per g.), it was 27% and, after further recrystallisation from ethanol (20 c.c. per g.), was 18% (Found, after drying in a vacuum-desiccator: I, 15·8. $C_{44}H_{55}N_2IS_2$ requires I, 15·8%). The dark green crystals melted gradually from 190° but there was no definite m. p.; at about 216° violent decomposition occurred. The absorption maximum of a methanolic solution lay at 5800 Å. Sensitisation was weak, with the maximum at 6100 Å.

*Bis*-2-[3-*ethylnaphtho*(2′ : 1′-4 : 5)*thiazole*]-β-*undecyltrimethincyanine Iodide*.—To the salt prepared from 2-undecylbenzoxazole (2·73 g.) and methyl sulphate were added 2-methylnaphtho(2′ : 1′-4 : 5)thiazole ethiodide and ethanol, and the hot mixture was treated with sodium dissolved in alcohol and boiled for 4 minutes. The washed product was boiled with ethanol (2 × 100 c.c.), and the crystals (0·85 g.) from the second extract seemed satisfactory. Those from the first (2·31 g.) were further boiled with ethanol (10 c.c., 2 × 25 c.c., 100 c.c.); the final of these products (0·75 g.) was boiled again with ethanol (2 × 20 c.c.), and the undissolved residue was added to the crystals from the second original extract. Their recrystallisation from ethanol (150 c.c.) gave a 15% yield (Found: I, 16·95. $C_{40}H_{47}N_2IS_2$ requires I, 17·0%). The green *dye* became brown at about 215° and had m. p. about 221° (decomp.). Its methanolic solution had its absorption maximum at 5800 Å. Weak sensitising extended to about 6500 Å with a maximum at 6200 Å.

Attempts similarly to prepare isomers of this dye and the preceding one, by use of 2-methylnaphtho(1′ : 2′-4 : 5)thiazole ethiodide, gave only 2% yields of recrystallised products.

*Bis*-2-(3-*ethylbenzoselenazole*)-β-*pentadecyltrimethincyanine Iodide*.—The washed dye, obtained by boiling an alcoholic mixture of 2-pentadecylbenzoxazole methomethylsulphate (4·55 g.) and 2-methylbenzoselenazole ethiodide with sodium dissolved in alcohol, was recrystallised from ethanol (80 c.c.), giving a 16% yield and a residue. A second recrystallisation (20 c.c. per g.) gave a 13% yield and no residue, and a third gave 11% (Found: after drying in a vacuum-desiccator: I, 16·1. $C_{36}H_{51}N_2ISe_2$ requires I, 15·95%). The green *dye* had no definite m. p.; it began to shrink at about 85°, was semi-molten below 100°, and frothed at about 150°. The absorption maximum of a methanolic solution was at 5600 Å. Weak sensitisation was from 5200 to 6300 Å, with the maximum at 5900 Å. This is example 6 of B.P. 439,359, where no properties are described.

2-p-*Dimethylaminostyrylbenzoxazole Ethotoluene*-p-*sulphonate* (XVII; R = CH:CH·$C_6H_4$·$NMe_2$, R″ = Et, X = $SO_3$·$C_6H_4$Me).—A mixture of 2-methylbenzoxazole ethotoluene-*p*-sulphonate (6·66 g., 1 mol.), *p*-dimethylaminobenzaldehyde (1 mol.), and triethylamine (0·1 mol.) in ethanol (10 c.c.) was boiled for 7 hours. The washed *dye* was boiled with benzene (20 c.c., then 2 × 10 c.c.), and the residue (38% yield) recrystallised from ethanol (10 c.c. per g.) (yield, 31%) (Found: S, 7·05. $C_{26}H_{28}O_4N_2S$ requires S, 6·9%). The vermilion crystals had m. p. 234° (decomp.), with shrinking from 228°. Their methanolic solution had its absorption maximum at 4970 Å. The dye sensitised a gelatino-chloride plate to 5800 Å, with a maximum at 5300 Å.

*Bis*-2-(3-*ethylbenzothiazole*)-β-p-*dimethylaminostyryltrimethincyanine Iodide* (XI).—To a hot mixture of 2-*p*-dimethylaminostyrylbenzoxazole ethotoluene-*p*-sulphonate (4·65 g., 1 mol.) and 2-methylbenzothiazole ethiodide (2 mols.) in ethanol (23 c.c.) was added a hot solution of sodium (3 atoms) in ethanol (23 c.c.), and boiling with stirring was continued for 10 minutes. The reaction mixture was filtered hot, residual solid being washed twice with hot ethanol before rejection. When the filtrate and washings were cooled, dye crystallised (1·64 g.). The filtrate from it was treated hot with a solution of potassium iodide (5 g.) in water (50 c.c.); after cooling, the dye iodide was filtered off and washed (0·84 g. obtained). The earlier crop of dye was dissolved in hot alcohol (20 c.c. per g.) and treated with a hot solution of potassium iodide (2 g. per g.) in water (20 c.c. per g. of dye), and the mixture boiled for 2 minutes; whilst hot, the dye iodide was filtered off and washed (0·74 g.), the total yield (1·58 g.) now amounting to 25%. On a second treatment with aqueous potassium iodide, with hot filtration, the yield dropped to 11% and, on recrystallisation from methanol (110 c.c. per g.), to 7% (Found: I, 20·25. Calc. for $C_{31}H_{32}N_2IS_2$: I, 19·9%). This sample, one prepared as below, and their mixture melted at 244° (decomp.), whereas Ogata gives 252° (decomp.) (*Bull. Chem. Soc. Japan*, 1936, **11**, 262).

Bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine bromide (2 g., 1 mol.), *p*-dimethyl-

aminobenzaldehyde (1·25 mols.), ethanol (12 c.c.), and piperidine (3 mols.) were boiled together for 25 minutes, which is Ogata's method, except that he used the less soluble iodide. The solution was treated hot with one of potassium iodide (4 mols.) in water (24 c.c.). The washed product was boiled with methanol (25 c.c.), and the residue (1·11 g.) recrystallised from methanol (125 c.c.) (yield, 27%; 19% after a second such recrystallisation) (Found: I, 19·85%). The methanolic solution had its absorption maximum at 5565 Å. Sensitisation was from 5200 to 6400 Å, with the maximum at 5900 Å.

*Bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine Chloride* (X; R = Me, X = Cl).—Bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine bromide (10 g., 1 mol.), dissolved in *m*-cresol (25 c.c.), was heated on the water-bath and stirred with freshly prepared silver chloride (3 mols.) for 1 hour, the lumps being broken up at intervals (Brooker and Eastman Kodak Co., U.S.P. 2,245,249—50/1941). The silver halide was filtered off hot and washed thrice with hot methanol. The cooled filtrate and washings were treated with ether (500 c.c.), and the dye chloride was filtered off and washed with ether. On recrystallisation of the crude product (10·09 g.) from methanol (5 c.c. per g.), the yield was 80%; when the filtrate was treated hot with its own volume of water containing potassium bromide, the yield of recovered dye bromide was 13%. A second recrystallisation of the *chloride* from methanol (10 c.c. per g.) gave a 64% yield of navy-blue crystals, with 9% recovery of dye bromide. Recrystallisation of a sample from acetic anhydride (10 c.c. per g.) brought the yield down to 48% (Found: Cl, 8·25. $C_{22}H_{23}N_2ClS_2$ requires Cl, 8·55%). The purple crystals had m. p. 224° (decomp.).

*Bis-2-(3-ethylbenzoselenazole)-β-methyltrimethincyanine chloride* was similarly prepared from bis-2-(3-ethylbenzoselenazole)-β-methyltrimethincyanine bromide (5 g.). The crude dye chloride (4·90 g.) was boiled with acetone (25 c.c.), and the residue (4·12 g., 90%) was recrystallised from methanol (20 c.c. per g.), giving a 61% yield of dark blue crystals. For analysis a sample was recrystallised from acetic anhydride (16 c.c. per g.), giving purple crystals, no definite m. p. but violent decomp. at about 245°. The absorption maximum of a methanolic solution was at 5560 Å (Found: N, 5·45; Cl, 6·9. $C_{22}H_{23}N_2ClSe_2$ requires N, 5·5; Cl, 6·95%). This and the preceding analysis were by Mr. F. H. Oliver, of the Imperial College. The corresponding iodide is known (Brooker, White, and Eastman Kodak Co., U.S.P. 2,058,406/1936) but no properties are described.

*Bis-2-(3-ethylbenzothiazole)-β-(2-2′-benzothiazolylvinyl)trimethincyanine Iodine* (XIII).—A mixture of bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine chloride (0·41 g., 1 mol.) and 2-formylbenzothiazole (1·5 mols.) in pyridine (3 c.c.) was heated for 5 minutes at 125—130°. The solution was treated hot with one of potassium iodide (2 mols.) in water (3 c.c.). From the cooled solution, tar separated and later solidified. It was filtered off and washed with aqueous pyridine and then with ether. To free this crude dye (0·46 g.) from pink impurity, it was dissolved in hot pyridine (20 c.c.) and treated with potassium iodide (0·92 g.) in hot water (20 c.c.). The purified, washed product (0·17 g.) was recrystallised from acetic anhydride (5 c.c.) (yield, 0·07 g., 11%). After a second recrystallisation from acetic anhydride (60 c.c. per g.), the yield was 5%. The dark green crystals had no definite m. p.; shrinking began at about 190° and the substance frothed at about 218° (Found: C, 54·7; H, 4·45. $C_{30}H_{26}N_2IS_2$ requires C, 55·3; H, 4·05%). A methanolic solution containing ammonia had its absorption maximum at 5630 Å. With one containing sulphuric acid, the absorption was in the same place but less intense.

When 2-anilomethylbenzothiazole (1·5 mols.) was used in place of 2-formylbenzothiazole, the crude washed dye iodide (0·47 g.) was not made bluer by dissolution in pyridine and treatment with potassium iodide solution, though the yield was reduced (to 0·22 g.). Recrystallisation from methanol (150 c.c. per g.) gave an 18% yield (0·09 g.) of crystals, having the same colour and solubility (Hamer, *J.*, 1928, 3160) as bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine iodide. Concentration of the filtrate gave a second crop (0·04 g.), containing some of the blue dye, and addition of ether to the filtrate from this gave a precipitate (0·02 g.), containing more of the bluer dye but still contaminated by the pink one. The first crop (0·09 g.), on recrystallisation from methanol, with addition of charcoal (0·02 g.) gave a 10% yield of the characteristic coppery crystals of bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine iodide, identified by appearance, colour of solution, m. p., and mixed m. p.

To carry out a preparation by the other method, a cold mixture of 2-(2-2′-benzthiazolylvinyl)benzoxazole methiodide (1·05 g., 1 mol.), 2-methylbenzothiazole ethiodide (2 mols.), and ethanol (5 c.c.) was treated with a hot solution of sodium (3 atoms) in ethanol (5 c.c.). The mixture was stirred and boiled for 1 minute and the purple solution treated hot with potassium iodide (4 mols.) in water (20 c.c.). The precipitated tar hardened on cooling and was filtered

off and well washed with water (1·37 g. left). It was dissolved in hot acetic anhydride (2 c.c.) and reprecipitated with ether (40 c.c.). The ether-washed tar was dissolved in hot methanol (4 + 1 c.c.), then precipitated with potassium iodide solution as before, and the solid filtered off and washed with water (0·52 g. obtained). Most of the red dye was removed by boiling the mixture with pyridine (25 c.c.), treating the solution with potassium iodide (1·04 g.) in hot water (25 c.c.), boiling it, and setting it aside to crystallise. The washed crystals (1½% yield) gave a blue alcoholic solution with only a tinge of pink, and this tinge disappeared on recrystallisation of the product from methyl alcohol (2 c.c.; yield ½%). These dark green crystals, the other sample, and their mixture showed gradual decomposition from below 200° and all frothed up together. The absorption maximum of a methanolic solution containing ammonia was the same as that of the other sample.

In test-tube experiments, no blue colour developed when bis-2-(3-ethylbenzoselenazole)-β-methyltrimethincyanine chloride was heated in pyridine with 2-anilomethylbenzoselenazole, but with 2-formylbenzoselenazole there was a transient blue colour. In an attempted condensation, the dye chloride (0·51 g., 1 mol.), 2-formylbenzoselenazole (1·5 mols.), and pyridine (3 c.c.) were heated together at 125—130° for 5 minutes and the solution was treated hot with potassium iodide (2 mols.) in water (3 c.c.). The washed dye was boiled with acetone (5 c.c., 2 × 10 c.c.), giving 42% recovery of dye iodide (0·25 g.), which was similar in colour to the original dye chloride. After recrystallisation from methanol (50 c.c.), the yield was 15%. In contact with the solution the crystals were brassy, but they were dark purple when dry; they decomposed violently at about 278° (Found: C, 44·05; H, 4·1. Calc. for $C_{23}H_{23}N_2ISe_2$: C, 43·95; H, 3·85%). The analysis and the colour in solution identified it as [bis-2-(3-ethylbenzoselenazole)]-β-methyltrimethincyanine iodide (Brooker, White, and Eastman Kodak Co., U.S.P. 2,058,406/1936).

In a similar experiment with 2-formylbenzothiazole, an 8% yield of recrystallised dye iodide was isolated, but not the more soluble blue dye, which appeared to be formed.

*Bis-2-(3-ethylbenzothiazole)-β-(2-2′-quinolylvinyl)trimethincyanine Iodide* (XIV; Y = S).—A mixture of bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine chloride (0·41 g., 1 mol.), aniloquinaldine (1·5 mols.), and pyridine (6 c.c.) was heated at 125—130° for 3 minutes, becoming blue. The solution was treated hot with potassium iodide (2 mols.) in water (6 c.c.). After cooling, the dye *iodide* was filtered off and washed with aqueous pyridine and then with ether. To remove pink contaminant, the crude dye (0·46 g.) was boiled with pyridine (15 c.c.) and treated with potassium iodide (0·92 g.), in hot water (15 c.c.). After cooling, the crystals were filtered off and washed (0·31 g. obtained). After recrystallisation from ethanol (125 c.c. per g.) and then from acetic anhydride (80 c.c. per g.) the yield was 27%. The dark green crystals had m. p. 216° (decomp.), with previous shrinking (Found: C, 59·75; H, 4·2. $C_{32}H_{28}N_2IS_2$ requires C, 59·5; H, 4·35%). A methanolic solution containing ammonia showed its maximum absorption at 5600 Å. In one containing sulphuric acid, this absorption band was less intense and there was a secondary band with its maximum at 6000 Å.

In a similar experiment in which 2-formylquinoline (1·5 mols.) took the place of aniloquinaldine, the colour of an alcoholic solution of the washed dye iodide (74%, 0·37 g.) was pink instead of blue. After recrystallisation from the amount of methanol (150 c.c. per g.) prescribed for bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine iodide (Hamer, *J.*, 1928, 3160), the yield was 52%. From the characteristic crystals, colour of the solution, m. p., and mixed m. p., the dye iodide was identified with that named.

According to the other method, a hot solution of sodium (3 atoms) in ethanol (5 c.c.) was added to a cold mixture of 2-(2-2′-quinolylvinyl)benzoxazole methiodide (1·04 g., 1 mol.), 2-methylbenzothiazole ethiodide (2 mols.), and ethanol (5 c.c.). The mixture was boiled and stirred for 1 minute and the purple solution treated with potassium iodide (4 mols.) in hot water (20 c.c.). The product (1·51 g.) was purified by precipitating it from hot acetic anhydride (2 c.c.) with ether (40 c.c.). The ether-washed tar was dissolved in hot methanol (4 + 1 c.c.) and precipitated with potassium iodide as before (0·55 g. obtained). Although this product contained far more red impurity than that prepared by the other method, a similar purification proved satisfactory. Thus it was boiled with pyridine (20 c.c.), treated with a hot solution of potassium iodide (1·1 g.) in water (20 c.c.), boiled, cooled, and left overnight. After the crystals had been filtered off and washed with aqueous pyridine and with water, they (0·02 g.) gave an alcoholic solution identical in colour with that of the material prepared by the other method. When the filtrate was treated with more potassium iodide (2·2 g.) in water (80 c.c.), some rather pinker dye (0·33 g.) was recovered. Another treatment of this in hot pyridine (5 c.c.) with potassium iodide (0·66 g.) in water (5 c.c.) gave a further yield (0·01 g.) of blue dye (total

2%). After recrystallisation from the same proportion of ethanol as used in the other method, similar green crystals were obtained, in 1% yield. They had the same m. p. and mixed m. p., and the absorption maximum (methanol containing ammonia) was the same.

In an attempt to prepare its ethiodide, the pure dye (0·29 g., 1 mol.) was heated at 100° with ethyl iodide (8 mols.) for 24 hours. After washing with ether, there was an 80% recovery (m. p., mixed m. p., and colour of solution). A sample heated with ethyl toluene-*p*-sulphonate (10 mols.) at 160—165° for 30 minutes appeared to decompose.

*Bis*-2-(3-*ethylbenzoselenazole*)-β-(2-2′-*quinolylvinyl*)*trimethincyanine Iodide* (XIV; Y = Se).—A mixture of bis-2-(3-ethylbenzoselenazole)-β-methyltrimethincyanine chloride (0·51 g., 1 mol.), aniloquinaldine (1·5 mols.), and pyridine (6 c.c.) was heated as in the preceding preparation and treated with potassium iodide. The washed crude dye (0·54 g.), dissolved in hot pyridine (12 c.c.), was treated with potassium iodide (0·54 g.) in hot water. The washed *product* (0·35 g.) was boiled with, and recrystallised twice from, methanol (yield, 6%) and then from acetic anhydride (40 c.c. per g.) (yield, 3%). The dark crystals frothed at about 227° (Found: C, 52·0; H, 4·5. $C_{33}H_{29}N_2ISe_2$ requires C, 51·9; H, 3·8%). A methanolic solution containing ammonia had its absorption maximum at 5700 Å. One containing sulphuric acid showed a weak maximum at 5640 and an inflexion at 6250 Å, and faded during exposure to light.

*Bis*-2-(3-*ethylbenzothiazole*)-β-(2-4′-*quinolylvinyl*)*trimethincyanine Iodide* (XV).—A mixture of bis-2-(3-ethylbenzothiazole)-β-methyltrimethincyanine chloride (0·41 g., 1 mol.), anilolepidine, and pyridine (3 c.c.) was heated as in other instances, and the solution treated hot with one of potassium iodide (2 mols.) in water (3 c.c.). The washed dye (0·48 g.) from two such preparations was boiled with pyridine (25 c.c.), treated with potassium iodide (1·44 g.) in hot water (25 c.c.), and the mixture boiled. The *dye* which crystallised on cooling was filtered off, washed, and dried (0·24 g. obtained). After recrystallisation from acetic anhydride (25 c.c.), the yield was 7% (0·09 g.). The dark purple crystals decomposed at about 241° (Found: C, 59·85; H, 4·2. $C_{33}H_{29}N_2IS_2$ requires C, 59·5; H, 4·35%). A methanolic solution containing ammonia had its absorption maximum at 5610 Å. One containing sulphuric acid showed less intense absorption, with the maximum at 5580 and inflexions at 6200 and 6500 Å: this solution became colourless overnight.

I am indebted to Dr. B. H. Carroll, of the Eastman Kodak Co., for the photographic tests, to Kodak Ltd. for the absorption data of the dinuclear trimethincyanines, to Mrs. A. I. Boston, of the Imperial College, for the other measurements of light absorption, and to Dr. E. A. Braude for his interest. I thank Kodak Ltd. for financing the work and especially Prof. R. P. Linstead, F.R.S., for providing facilities which have made its completion possible.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
SOUTH KENSINGTON, LONDON, S.W. 7. [*Received, April 17th*, 1952.]

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## 614. *The Chemistry of Extractives from Hardwoods. Part VIII.* The Isolation of* 5 : 4′-*Dihydroxy*-7-*methoxy*isoflavone (*Prunetin*) *from the Heartwood of* Pterocarpus angolensis *and a Synthesis of* 7 : 4′-*Dihydroxy*-5-*methoxy*isoflavone *hitherto known as Prunusetin.*

By F. E. KING and L. JURD.

A further extractible constituent of *Pterocarpus angolensis* (see *J.*, 1952, 96, 1920) is shown to be 5 : 4′-dihydroxy-7-methoxy*iso*flavone (prunetin).

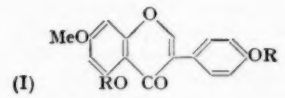
By starting from genistein, the isomeric 7 : 4′-dihydroxy-5-methoxy*iso*flavone has been synthesised for the first time. It is not identical with the natural product prunusetin (Chakravarti and Bhar, *J. Indian Chem. Soc.*, 1945, **22**, 301) which on the evidence now available cannot be distinguished from prunetin.

ALCOHOLIC extracts of the commercial timber muninga (*Pterocarpus angolensis*), from which 6 : 4′-dihydroxy-5 : 7-dimethoxy*iso*flavone (muningin) and 2 : 4-dihydroxyphenyl 1-*p*-methoxyphenylethyl ketone (angolensin) have recently been obtained, also contain 5 : 4′-dihydroxy-7-methoxy*iso*flavone (prunetin) (I; R = H). On prolonged methylation

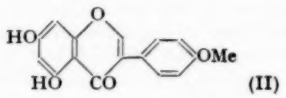
* Part VII, *J.*, 1952, 1920.

the new product formed a dimethyl ether shown by direct comparison with an authentic specimen to be 5 : 7 : 4′-trimethylgenistein (I; R = Me). Thereafter, its identity with genistein 7-methyl ether (I; R = H), first isolated by Finnemore (*Pharm. J.*, 1910, **31**, 604) from the bark of a *Prunus* species, was evident from the correspondence in melting points of their respective mono- and di-acetates, monomethyl ethers, and monomethyl ether acetates. Prunetin diethyl ether has a higher melting point than that recorded for a compound of that description recently synthesised (Narasimhachari and Seshadri, *Proc. Indian Acad. Sci.*, 1950, **32**, 256).

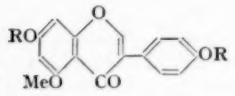
Of the three possible monomethyl ethers of genistein, the structures of two, namely, prunetin (I; R = H) and biochanin-A (5 : 7-dihydroxy-4′-methoxy*iso*flavone) (II), extracted by Sidiqui from germinated Chana grain (*J. Sci. Ind. Res., India*, 1945, **4**, 68; Bose and Siddiqui, *ibid.*, p. 231) have been firmly established by syntheses (Iyer, Shah, and Venkataraman, *Current Sci.*, 1949, **18**, 404; Shriner and Hull, *J. Org. Chem.*, 1945, **10**, 288,



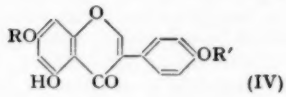
(I)



(II)



(III)



(IV)

respectively). The remaining *O*-monomethylgenistein (III; R = H) has not so far been obtained synthetically but is reported to occur in the bark of *Prunus puddum* (Chakravarti and Bhar, *J. Indian Chem. Soc.*, 1945, **22**, 301) and has been called prunusetin. The reported melting points of prunusetin (237—238°) and of its diacetate (220—222°), which is the sole characteristic derivative prepared by Chakravarti and Bhar, do not appreciably differ from those recorded by Finnemore (*loc. cit.*) for prunetin (242° corr.) and prunetin diacetate (224—226°); consequently, there was at first some doubt as to the precise orientation of the dihydroxymethoxy*iso*flavone derived from *Pterocarpus angolensis* (m. p. 237—238°; diacetate, 222—225°). However, the intense ferric colour reaction exhibited by the muninga extractive, a characteristic property of the chelated 5-hydroxyl group, appeared decisively to exclude the structure (III; R = H) and, the properties of (II) being obviously different, it was thus possible to identify the heartwood constituent as prunetin (I; R = H).

Chakravarti and Bhar record for their dihydroxymethoxy*iso*flavone a similar well-marked ferric reaction, but nevertheless regard it as (III; R = H) largely from its formation of a dimethyl ether (I; R = Me), in apparent contrast to prunetin from which Finnemore (*loc. cit.*) obtained only a monomethyl derivative. The Indian authors were apparently unaware that the methylation conditions they employed do not correspond with those recorded by Finnemore, and, when we applied the two different procedures to the dihydroxymethoxy*iso*flavone from muninga, prunetin monomethyl ether (5-hydroxy-7 : 4′-dimethoxy*iso*flavone) (IV; R = R′ = Me) was obtained on methylation with methyl iodide and sodium methoxide in methanol (Finnemore's method), whereas the reagent used by Chakravarti and Bhar (methyl iodide, potassium carbonate) afforded the dimethyl ether (I; R = Me). The principal evidence differentiating the supposed 7 : 4′-dihydroxy-5-methoxy*iso*flavone of Chakravarti and Bhar from its isomer prunetin is thereby invalidated. Its identification as the 5-methyl ether of genistein (III; R = H) was further discredited by the completion of a synthesis of this compound which has shown that the new *iso*flavone has m. p. 302° (diacetate, m. p. 169·5°) and exhibits only a very feeble ferric reaction. The so-called prunusetin of Chakravarti and Bhar on the evidence now available is indistinguishable from prunetin (I; R = H).

Genistein was the starting point for our synthesis of the new *iso*flavone (III; R = H). Considerable quantities were available in the form of its 4′-methyl ether, biochanin-A (II), which had been obtained during large-scale extractions of the heartwood of *Ferreirea*

*spectabilis* (F. E. King, M. F. Grundon, and K. G. Neill, forthcoming publication). Genistein, obtained by the demethylation of (II), under prolonged treatment with benzyl chloride and sodium carbonate or sodium ethoxide gave a dibenzyl ether, chelation of the 5-hydroxyl group limiting the reaction to the formation of 7 : 4′-dibenzyloxy-5-hydroxy-*iso*flavone (IV; R = R′ = $CH_2Ph$). Further alkylation with methyl iodide and potassium carbonate afforded the corresponding methyl ether (III; R = $CH_2Ph$) from which the required 5-methylgenistein (III; R = H) was obtained by debenzylation in a solution of hydrochloric and acetic acids.

The availability of biochanin-A was utilised in a synthesis of 7-hydroxy-5 : 4′-dimethoxy*iso*flavone formerly obtained by hydrolysis of trimethylgenistin (genistein-7-D-glucoside trimethyl ether) and by partial demethylation of 5 : 7 : 4′-trimethylgenistein (Walz, *Annalen*, 1931, **489**, 118). The *iso*flavone (II) was monobenzylated and the 7-benzyl ether then methylated, the resulting 7-benzyloxy-5 : 4′-dimethoxy*iso*flavone being hydrolysed under mild conditions to a product identical with that obtained by Walz.

## Experimental

*Isolation of Prunetin* (I; R = H).—After removal of muningin from an alcoholic extract of the powdered wood by crystallisation from methanol as already described (*J.*, 1952, 98), the dark resinous solid (750 g.) obtained on evaporating the solvent was finely powdered and heated under reflux with acetone (2·5 l.) for 1 hour. Insoluble material was then removed by filtration and the filtrate concentrated to approx. 1 l. and diluted with ether (4 l.). When the dark red granular precipitate containing a further small amount of muningin had been collected, the dark acetone–ether solution was twice washed with N-sodium hydroxide (total 1 l.). The use of this limited quantity of alkali enabled the prunetin to be retained by the mixture of organic solvents which was then washed with water, dried ($MgSO_4$), and evaporated. The yellow oily residue was dissolved in an equal weight of hot methanol, the *iso*flavone separating overnight as a pale yellow solid (6·2 g.), m. p. 236°. Recrystallisation from methanol gave pale cream-coloured silky needles m. p. 237—238° (Found: C, 67·3; H, 4·5; OMe, 9·8. Calc. for $C_{16}H_{12}O_5$: C, 67·6; H, 4·3; OMe, 10·9%). In alcoholic solution it gave a deep brown-purple colour with ferric chloride. With acetic anhydride and sodium acetate on a steam-bath for 45 minutes diacetylprunetin was formed, and it crystallised from methanol in flat thick rods, m. p. 222·5° (Found: C, 65·0; H, 4·4; OMe, 8·7. Calc. for $C_{20}H_{16}O_7$: C, 65·2; H, 4·4; 1OMe, 8·4%).

A solution of prunetin (0·1 g.) in pyridine (0·4 c.c.) briefly warmed with acetic anhydride (0·25 c.c.) and poured into water gave 4′-acetoxy-5-hydroxy-7-methoxy*iso*flavone, which when successively crystallised from benzene–light petroleum and methanol formed fine needles, m. p. 187° (Finnemore, *loc. cit.*, records 190°) (Found: C, 66·2; H, 4·5; OMe, 7·9. Calc. for $C_{18}H_{14}O_6$: C, 66·2; H, 4·3; 1OMe, 9·5%). In alcoholic solution the ferric reaction was intense red-violet.

*Prunetin Dimethyl Ether* (I; R = Me).—The action of methyl iodide (5 c.c.) and potassium carbonate (3 g.) on prunetin (1 g.) in refluxing acetone for 24 hours gave 5 : 7 : 4′-trimethoxy*iso*flavone which crystallised from benzene–light petroleum in thin plates (0·98 g.), m. p. 161·5° alone or mixed with a specimen prepared from biochanin-A. From the oxidation of prunetin dimethyl ether with nitric acid on a steam-bath, 4-methoxy-3-nitrobenzoic acid was isolated on the addition of water. Recrystallisation gave needles, m. p. and mixed m. p. 192—193°.

Unlike prunetin, the dimethyl ether dissolved in hot concentrated hydrochloric acid and deposited, on cooling, yellow needles of the readily hydrolysed *iso*flavone hydrochloride.

Hydrolysis of the dimethyl ether (0·2 g.) with potassium hydroxide (1 g.) in water (2 c.c.) and alcohol (20 c.c.) under reflux for 2 hours afforded 2-hydroxy-4 : 6-dimethoxyphenyl 4-methoxybenzyl ketone, which after evaporation of the alcohol and acidification was isolated by ether-extraction. Crystallised from light petroleum it had m. p. 87° (lit., 88—89°) (Found: C, 67·7; H, 6·1. Calc. for $C_{17}H_{18}O_5$: C, 67·5; H, 6·0%).

*5-Hydroxy-7 : 4′-dimethoxyisoflavone* (*Prunetin Monomethyl Ether*) (IV; R = R′ = Me).—(i) A mixture of prunetin (0·25 g.), methyl sulphate (0·12 g., 1·1 mols.), anhydrous potassium carbonate (1 g.), and acetone (25 c.c.) was heated under reflux for 5 hours. The product, precipitated after filtration and on the addition of water, crystallised from methanol, whereupon prunetin monomethyl ether was obtained in flat needles (0·18 g.), m. p. 144° (Found: C, 68·8; H, 5·2; OMe, 19·6. Calc. for $C_{17}H_{14}O_5$: C, 68·4; H, 4·7; 2OMe, 20·8%). With alcoholic ferric chloride the ether gave an intense red colour.

(ii) When excess of ethereal diazomethane was added to a suspension of prunetin (50 mg.) in methanol a clear solution was obtained from which prunetin monomethyl ether began to separate. Isolated by evaporation of the solvents and crystallised from methanol it had m. p. 138—139°.

(iii) Repetition of the procedure described by Finnemore (*loc. cit.*), using methyl iodide in methanolic sodium methoxide, though less satisfactory than the foregoing methods, afforded the monomethyl ether, m. p. 138—139° after several crystallisations from methanol.

When heated with sodium acetate and acetic anhydride on a steam-bath for 30 minutes, the monoacetate (5-acetoxy-7 : 4′-dimethoxy*iso*flavone) was obtained as diamond-shaped plates (from methanol), m. p. 202° (Found : C, 66·7; H, 4·9; OMe, 17·6. Calc. for $C_{19}H_{16}O_6$ : C, 67·0; H, 4·7; 2OMe, 18·2%).

5 : 4′-*Diethoxy*-7-*methoxy*iso*flavone* (*Prunetin Diethyl Ether*) (I; R = Et).—A mixture of prunetin (0·4 g.), ethyl iodide (3 c.c.), potassium carbonate (8 g.), and acetone (100 c.c.) was heated under reflux for 18 hours. The solution was then filtered and evaporated and the oily residue shaken with N-sodium hydroxide. The insoluble solid was collected and crystallised from methanol and, after further recrystallisations from benzene–light petroleum, prunetin diethyl ether was obtained in needles, m. p. 126° (Found : C, 70·6; H, 6·0. Calc. for $C_{20}H_{20}O_5$ : C, 70·5; H, 5·9%). For the product similarly prepared from 4′-ethoxy-5-hydroxy-7-methoxy-*iso*flavone Narasimhachari and Seshadri (*loc. cit.*) found m. p. 116—117°.

7 : 4′-*Dibenzyloxy*-5-*hydroxy*iso*flavone* (IV; R = R′ = $CH_2Ph$).—(*a*) A mixture of 5 : 7 : 4′-trihydroxy*iso*flavone (5 g.), benzyl chloride (8 c.c.), anhydrous sodium carbonate (15 g.), and sodium iodide (0·5 g.) in acetone (200 c.c.) was heated under reflux for 48 hours. When the solid had been removed, the acetone solution was concentrated to 20 c.c. and mixed with methanol (20 c.c.); the precipitate which slowly separated was then combined with the residue of sodium salts from the benzylation, and the whole shaken with dilute aqueous sodium hydroxide. The insoluble product was crystallised from acetone which deposited 7 : 4′-*dibenzyloxy*-5-*hydroxy*iso*flavone* as very faintly yellow crystals (3·8 g.), m. p. 196°. For analysis the compound was recrystallised from benzene and formed shining rectangular plates, m. p. 197° (Found : C, 77·3; H, 4·6. $C_{29}H_{22}O_5$ requires C, 77·3; H, 4·9%). An alcoholic solution gave a red colour with ferric chloride.

(*b*) The 5 : 7 : 4′-trihydroxy*iso*flavone (2·5 g.) and benzyl chloride (5 c.c.) were mixed with a solution of sodium (0·5 g.) in dry ethanol (25 c.c.). After boiling under reflux for 3 hours, the mixture was diluted with water, and the excess of benzyl chloride removed by steam-distillation. The sticky solid was collected and dissolved in boiling alcohol (15 c.c.). The product which separated on cooling was treated with N-sodium hydroxide, and the solid which remained undissolved was recovered, dried, and recrystallised from benzene. The dibenzyl ether (1·07 g.) had m. p. and mixed m. p. 197°.

Acetylation with sodium acetate and boiling acetic anhydride for 30 minutes and treatment with water gave 5-*acetoxy*-7 : 4′-*dibenzyloxy*iso*flavone* which crystallised from methanol and from benzene–light petroleum in minute needles, m. p. 197—198° (Found : C, 76·0; H, 5·0. $C_{31}H_{24}O_6$ requires C, 75·6; H, 4·9%).

7 : 4′-*Dibenzyloxy*-5-*methoxy*iso*flavone* (III; R = $CH_2Ph$).—The dibenzylhydroxy*iso*flavone (3·8 g.) was methylated with methyl iodide (5 c.c.) and potassium carbonate (15 g.) in acetone (150 c.c.) under reflux for 24 hours. The filtered solution was evaporated and the solid obtained after the addition of water was crystallised from ethanol (yield, 3·4 g.; m. p. 163°). Crystallisation from benzene–light petroleum or acetone–methanol gave 7 : 4′-*dibenzyloxy*-5-*methoxy*-iso*flavone* as prisms, m. p. 164° (Found : C, 77·6; H, 5·3; OMe, 7·1. $C_{30}H_{24}O_5$ requires C, 77·6; H, 5·2; OMe, 6·7%).

7 : 4′-*Dihydroxy*-5-*methoxy*iso*flavone* (III; R = H).—A solution of the dibenzyloxymethoxy-*iso*flavone (3·3 g.) in acetic acid (200 c.c.) and concentrated hydrochloric acid (100 c.c.) was heated on a steam-bath for 1½ hours. When cooled and neutralised with aqueous sodium hydroxide it gave a precipitate of 7 : 4-*dihydroxy*-5-*methoxy*iso*flavone* which crystallised from methanol in needles (1·7 g.), m. p. after previous darkening, 302° (Found : C, 67·7; H, 4·6; OMe, 10·4. $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·3; OMe, 10·9%). An alcoholic solution of the *iso*flavone with ferric chloride was coloured yellow. By heating the compound with sodium acetate and acetic anhydride at 100° for 30 minutes, 7 : 4′-*diacetoxy*-5-*methoxy*iso*flavone* was obtained, and it crystallised from methanol or benzene–light petroleum in shining rectangular plates, m. p. 169·5° (Found : C, 65·1; H, 4·6; OMe, 8·2. $C_{20}H_{16}O_7$ requires C, 65·2; H, 4·4; OMe, 8·4%).

7-*Benzyloxy*-5-*hydroxy*-4′-*methoxy*iso*flavone*.—Biochanin-A (2 g.) and benzyl chloride (3 c.c.) were added to a solution of sodium (0·2 g.) in ethanol (15 c.c.) which was then heated under

reflux for 2 hours. After dilution with water and steam-distillation, the residual liquid was made alkaline with 2N-sodium hydroxide (10 c.c.). The remaining solid was collected, washed with alcohol, and crystallised from acetone–methanol. *7-Benzyloxy-5-hydroxy-4′-methoxyisoflavone* (1·2 g.) was thus obtained in pale cream-coloured plates, m. p. 190° (Found: C, 73·7; H, 4·8. $C_{23}H_{18}O_5$ requires C, 73·8; H, 4·8%).

*7-Benzyloxy-5: 4′-dimethoxyisoflavone.*—The foregoing monohydroxyisoflavone (0·95 g.) was methylated in acetone with methyl iodide and potassium carbonate in the customary way, and after one crystallisation from ethanol the resulting *7-benzyloxy-5: 4′-dimethoxyisoflavone* (0·7 g.) had m. p. 148°. By further crystallisations from methanol–acetone and ethanol it was obtained in prisms, m. p. 150° (Found: C, 74·5; H, 5·1; OMe, 16·6. $C_{24}H_{20}O_5$ requires C, 74·2; H, 5·2; 2OMe, 16·0%).

*5: 4′-Dimethoxy-7-hydroxyisoflavone.*—The dimethoxybenzyl ether (0·5 g.) was heated on a steam-bath with acetic acid (25 c.c.) and concentrated hydrochloric acid (12 c.c.) for 80 minutes. The cooled solution was neutralised with aqueous sodium hydroxide, and the precipitate collected and crystallised from acetone–methanol. 5: 4′-Dimethoxy-7-hydroxyisoflavone separated in faintly pink prisms (0·15 g.), m. p. 294°. Walz (*loc. cit.*) records m. p. 290—293° (Found: C, 68·5; H, 4·7; OMe, 20·9. Calc. for $C_{17}H_{14}O_5$: C, 68·4; H, 4·7; 2OMe, 20·8%).

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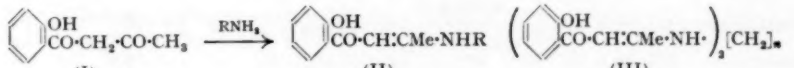
## 615. *Characterisation of Primary Aliphatic Amines by Reaction with o-Acetoacetylphenol and by Paper Chromatography.*

By WILSON BAKER, J. B. HARBORNE, and W. D. OLLIS.

Primary aliphatic amines may be characterised by reaction with *o*-acetoacetylphenol (I) to give crystalline *o*-β-alkylaminocrotonylphenols (II) which show an intense characteristic greenish-yellow fluorescence in ultra-violet light. Amines may be satisfactorily separated by paper chromatography, and the primary amines located as fluorescent spots after spraying with a solution of *o*-acetoacetylphenol.

IT was observed by Baker and Butt (*J.*, 1949, 2142) that *o*-acetoacetylphenol (I) reacted with benzylamine and with methylamine to give yellow products which showed vivid greenish-yellow fluorescence in ultra-violet light. These products were regarded as *o*-β-alkylaminocrotonylphenols (II), and this structure has since been proved by Baker, Harborne, and Ollis (*J.*, 1952, 1294). The enamine structure (II) is preferred to that of the tautomeric ketimine because the extended conjugated system in the former is more consistent with the colour and fluorescence of these substances. This reaction is now shown to be general for primary aliphatic amines (excluding zwitterions) and may be used for their characterisation.




(I) (II) (III)

Many primary aliphatic amines, including some of biological importance such as tyramine and tryptamine (see Experimental), react with ethanolic *o*-acetoacetylphenol (I) at room temperature. Aliphatic primary diamines also react to give the expected bis-condensation products (III). All these derivatives are stable, easily purified by recrystallisation, and yield copper complexes with cupric acetate (Baker, Harborne, and Ollis, *loc. cit.*). They are well suited for the characterisation of primary aliphatic amines. *o*-Acetoacetylphenol does not react with secondary amines, primary aromatic amines, or amino-acids.

2-Acetoacetyl-5-methoxyphenol and 2-acetoacetyl-4-methylphenol (Baker and Butt, *loc. cit.*) react similarly with primary aliphatic amines to give fluorescent products, and it is clear that this is a general reaction of ω-acetyl-*o*-hydroxyacetophenones. The very ready formation of the brilliantly fluorescent *o*-β-alkylaminocrotonylphenols (II) has led to a method for locating primary aliphatic amines in paper chromatography. Amines have been separated by using *n*-butanol–acetic acid–water as the mobile phase, and after development the paper was dried, sprayed with a 1% solution of *o*-acetoacetylphenol in *n*-butanol, and the amines located by the fluorescent spots observed in ultra-violet light. This method gave good results with biologically important amines such as tyramine and tryptamine, and with various sympathomimetic amines of pharmacological interest including noradrenaline. Some naturally occurring amines have been identified previously by paper chromatography including, for example, histamine (Urbach, *Proc. Soc. Exp. Biol.*, 1948, **68**, 430; 1949, **70**, 146; 1949, **72**, 626; Block, *Anal. Chem.*, 1950, **22**, 1327) and noradrenaline (James, *Nature*, 1948, **161**, 851; Goldenberg, Faber, Alston, and Chargaff, *Science*, 1949, **109**, 534; Glazko and Dill, *Nature*, 1951, **168**, 32; Crawford and Outschoorn, *Brit. J. Pharmacol.*, 1951, **16**, 8). Recently, a method for the identification of the terminal amino-acids in proteins by conversion into amino-alcohols has been reported (Fromageot, Jutisz, Meyer, and Penasse, *Biochem. Biophys. Acta*, 1950, **6**, 283); it is suggested that these amino-alcohols might be characterised by reaction with *o*-acetoacetylphenol.

The $R_F$ values of different amines on paper vary as expected according to their hydrophilic character. As the homologous series of primary aliphatic amines is ascended, so the $R_F$ value increases, and the $R_F$ value of diamines or of monoamines containing hydrophilic substituents is less than the $R_F$ values of corresponding unsubstituted amines.

| Amine | Product : R in (II) | Recryst. from : ‡ | M. p. | Yield, % | Formula | Found, % : C | H | N | Required, % : C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Methyl- ..... | * Methyl- | *e* | 101° | 24 | $C_{11}H_{13}O_2N$ | — | — | — | — | — | — |
| Ethyl- ..... | *Ethyl-* | *a* | 97·5 | 42 | $C_{12}H_{15}O_2N$ | 70·5 | 7·6 | 6·8 | 70·3 | 7·3 | 6·8 |
| *n*-Propyl- ..... | n-*Propyl-* | *b* | 77—78 | 63 | $C_{13}H_{17}O_2N$ | 71·0 | 7·5 | 6·4 | 71·2 | 7·8 | 6·4 |
| *iso*Propyl ..... | iso*Propyl-* | *a* | 89 | 63 | $C_{13}H_{17}O_2N$ | 71·3 | 7·6 | 6·3 | ,, | ,, | ,, |
| *n*-Butyl- ..... | n-*Butyl-* | *a* | 106 | 74 | $C_{14}H_{19}O_2N$ | 71·9 | 7·9 | 6·1 | 72·1 | 8·1 | 6·0 |
| *iso*Butyl- ..... | iso*Butyl-* | *a* | 111—112 | 27 | $C_{14}H_{19}O_2N$ | 72·2 | 7·8 | 6·0 | ,, | ,, | ,, |
| *n*-Hexyl- ..... | * *n*-Hexyl- | *a* | 95—96 | 67 | $C_{16}H_{23}O_2N$ | — | — | — | — | — | — |
| *cyclo*Hexyl- ..... | cyclo*Hexyl-* | *a* | 92 | 41 | $C_{16}H_{21}O_2N$ | 74·1 | 8·1 | 5·5 | 74·1 | 8·1 | 5·4 |
| Benzyl- ..... | * Benzyl- | *a* | 124 | 53 | $C_{17}H_{17}O_2N$ | — | — | — | — | — | — |
| *n*-Octyl- ..... | n-*Octyl-* | *a* | 95—97 | 75 | $C_{18}H_{27}O_2N$ | 74·8 | 9·9 | 4·7 | 74·8 | 9·3 | 4·8 |
| Ethanolamine ... | 2′-*Hydroxyethyl-* | *f* | 122 | 36 | $C_{12}H_{15}O_3N$ | 65·4 | 6·7 | 6·2 | 65·2 | 6·8 | 6·3 |
| † Tyramine ..... | *Tyramino-* | *c* | 147 | 54 | $C_{18}H_{19}O_3N$ | 72·8 | 6·4 | 4·7 | 72·7 | 6·4 | 4·7 |
| † Tryptamine ... | *Tryptamino-* | *a* | 167 | 30 | $C_{20}H_{20}O_2N_2$ | 74·7 | 6·2 | 8·8 | 75·0 | 6·3 | 8·7 |
| Diamine | | | | | | | | | | | |
| Ethylene- ..... | (III; $n = 2$) | *d* | 183 | 55 | $C_{22}H_{24}O_4N_2$ | 69·5 | 6·3 | 7·4 | 69·7 | 6·3 | 7·4 |
| Trimethylene- | (III; $n = 3$) | *d* | 171—172 | 13 | $C_{23}H_{26}O_4N_2$ | — | — | 7·3 | — | — | 7·1 |
| Tetramethylene- | (III; $n = 4$) | *d* | 210 (decomp.) | 44 | $C_{24}H_{28}O_4N_2$ | — | — | 7·2 | — | — | 6·9 |
| Pentamethylene- | (III; $n = 5$) | *d* | 190—191 | 34 | $C_{25}H_{30}O_4N_2$ | 70·9 | 6·9 | 6·7 | 71·1 | 7·1 | 6·6 |
| Hexamethylene- | (III; $n = 6$) | *d* | 183—184 | 64 | $C_{26}H_{32}O_4N_2$ | — | — | 6·7 | — | — | 6·4 |
| 1 : 2-Diaminopropane ..... | (cf. III) | *e* | 187 | 40 | $C_{23}H_{26}O_4N_2$ | 69·8 | 6·4 | 7·4 | 70·0 | 6·6 | 7·1 |

* These derivatives have been described previously, the first two by Baker and Butt (*loc. cit.*), and the last by Baker, Harborne, and Ollis (*loc. cit.*).

† For the reaction with *o*-acetoacetylphenol, the amine was liberated from its hydrochloride by treating a solution of the latter in ethanol with ethanolic sodium ethoxide. Tyramine, which is insoluble in cold ethanol, was treated with *o*-acetoacetylphenol in hot ethanol.

‡ Solvents used for recrystallisation : *a*, ethanol; *b*, methanol; *c*, aqueous ethanol; *d*, benzene; *e*, benzene–light petroleum; *f*, aqueous methanol.

## Experimental

M. p.s are uncorrected. Analyses are by Drs. Weiler and Strauss, Oxford, and Mr. W. M. Eno, Bristol.

*Reaction of o-Acetoacetylphenol with Primary Aliphatic Amines. Formation of o-β-Alkylaminocrotonylphenols* (II).—The following general method was used. The amines (1·1 equiv.) alone or dissolved in ethanol were added to an ethanolic solution of *o*-acetoacetylphenol (1 equiv.; Wittig, *Annalen*, 1926, **446**, 169) at room temperature; the solutions immediately became yellow-green and were strongly fluorescent in ultra-violet light. After a few hours the *o*-β-alkylaminocrotonylphenols began to crystallise and after 2 days the solid was collected and crystallised from the appropriate solvent. The products were obtained as pale green or greenish-yellow prisms or needles which showed a very strong greenish-yellow fluorescence in ultra-violet light.

*Reaction of 2-Acetoacetyl-5-methoxyphenol with Methyl-, Ethyl-, and Benzyl-amines.*—The amines were treated with 2-acetoacetyl-5-methoxyphenol as in the previous cases and the products were recrystallised from ethanol. The yields are given in parentheses. Methylamine gave *5-methoxy-2-β-methylaminocrotonylphenol* (60%), pale yellow plates, m. p. 157° (Found: C, 65·6; H, 7·0; N, 6·5. $C_{12}H_{15}O_3N$ requires C, 65·2; H, 6·8; N, 6·3%). Ethylamine gave *2-β-ethylaminocrotonyl-5-methoxyphenol* (83%), pale yellowish-green plates, m. p. 112·5° (Found: C, 66·2; H, 7·2; N, 6·0. $C_{13}H_{17}O_3N$ requires C, 66·4; H, 7·2; N, 6·0%). Benzylamine gave *2-β-benzylaminocrotonyl-5-methoxyphenol* (96%), pale green needles, m. p. 133° (Found: C, 72·6; H, 6·1; N, 4·7. $C_{18}H_{19}O_3N$ requires C, 72·7; H, 6·4; N, 4·7%). These compounds were vividly fluorescent in ultra-violet light.

*Paper Partition Chromatography of Primary Amines.*—The following amines have been separated on Whatman No. 1 filter-paper at room temperature, butanol–acetic acid–water mixture being used as the mobile phase. After running overnight the paper was dried, sprayed with a 1% solution of *o*-acetoacetylphenol in *n*-butanol, dried again, and examined in ultra-violet light. The amines were located by well-formed, brilliantly fluorescent spots. A list of the amines used is given below; the $R_F$ values are recorded in parentheses.

The solvents used for the development of the chromatograms were either the upper layer obtained by mixing *n*-butanol (5 parts), acetic acid (1 part), and water (4 parts), or *n*-butanol (2 parts), acetic acid (1 part), and water (1 part).

The first solvent mixture being used, the following amines were separated: benzylamine (0·70); *n*-hexylamine (0·80); *n*-heptylamine (0·83); *n*-octylamine (0·85); *n*-decylamine (0·87). By using the second solvent mixture the following amines were separated. Those marked thus * (below) were put on the paper as either hydrochlorides or sulphates.

*Monoamines*: Tryptamine* (0·39); tyramine* (0·58); benzylamine (0·66); 2-hydroxyethylamine (0·48); 1-phenylethylamine (0·72); 2-phenylethylamine (0·72); 2-aminoheptane (0·76).

*Diamines*: Ethylenediamine (0·39); trimethylenediamine (0·39); hexamethylenediamine (0·48); decamethylenediamine (0·62); 2-amino-2′-hydroxydiethylamine (0·42).

| *Sympathomimetic amines and analogues*: | | $R_F$ |
|---|---|---|
| * (3 : 4)$(HO)_2C_6H_3$·CH(OH)·$CH_2$·$NH_2$ ..... | Noradrenaline (L-Arterenol) | 0·34 |
| * (3 : 4)$(HO)_2C_6H_3$·CH(OH)·CHMe·$NH_2$ | (Cobefrin) | 0·42 |
| * (3 : 4)$(HO)_2C_6H_3$·CH(OH)·CHEt·$NH_2$ | (Butanefrine) | 0·50 |
| *p*-$HO·C_6H_4$·CH(OH)·CHMe·$NH_2$ ..... | — | 0·53 |
| * *p*-$HO·C_6H_4$·$CH_2$·CHMe·$NH_2$ ..... | (Paredrine) | 0·64 |
| * Ph·CH(OH)·CHMe·$NH_2$ ..... | (Propadrine) | 0·68 |
| * Ph·$CH_2$·CHMe·$NH_2$ ..... | (Dexedrine) | 0·74 |

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The University, Bristol. [*Received, May 3rd*, 1952.]

## 616. *Phase Equilibria in Sulphonic Acid–Water Systems.*

By Duncan Taylor and George C. Vincent.

Solubility and vapour-pressure relations for the systems $X{\cdot}C_6H_4{\cdot}SO_3H$–$H_2O$, where X = H, *o*-Me, *p*-Me, *p*-$NO_2$, *p*-F, *p*-Cl, *p*-Br, have been measured, 20 hydrates identified, and several heats of solution and of dissociation calculated. Dingemans's treatment of three-phase data at transition points of anhydrous polymorphs has been extended to inter-hydrate transitions, thus providing heats of dissociation independently of dissociation pressures. The results indicate a greater stability of monohydrates relatively to higher hydrates, in agreement with probable ionic structures for the former.

Williamson (*Trans. Faraday Soc.*, 1944, **40**, 421), in calculating heats of solution from solubility and activity data, took his examples largely from inorganic compounds. The same applies to the work of Dingemans and his co-workers (*Rec. Trav. chim.*, 1943, **62**, 625, 653) on three-phase equilibria in two-component systems and that of Pedersen (*Acta Chem. Scand.*, 1949, **3**, 65) on the calculation of hydrate dissociation pressures from saturated-solution vapour pressures. The following investigation involving benzene-, toluene-*o*- and -*p*-, and *p*-nitro-, *p*-fluoro-, *p*-chloro-, and *p*-bromo-benzene-sulphonic acids was carried out as a specific application, and in certain cases test, of the above theoretical work in aqueous systems of highly soluble organic compounds. It was desired first to establish from solubility measurements which hydrates were formed, and then to determine vapour-pressure data for both saturated and unsaturated solutions to allow (1) calculation of heats of solution by Williamson's procedure, (2) comparison of the observed temperatures of pressure maxima on the three-phase curves with those calculated from an equation of Dingemans *et al.*, and (3) calculation of hydrate dissociation pressures, and hence heats of dissociation, from Pedersen's equations. It was also proposed to extend Dingemans's calculations of heats of transition of polymorphs, using three-phase data, to include the case of inter-hydrate transitions. Heats of dissociation would thus be available without the need for solid–vapour equilibrium data, which are difficult to determine, or metastable equilibrium data as required in Pedersen's calculation. Furthermore, in view of a possible analogy with sulphuric and perchloric acids, for whose monohydrates ionic structures have been proposed (Volmer, *Annalen*, 1924, **440**, 200; Richards and Smith, *Trans. Faraday Soc.*, 1951, **47**, 1261), direct determinations of monohydrate dissociation pressures were planned to confirm their expected high stability. Little appears to have been published about $X{\cdot}C_6H_4{\cdot}SO_3H$–$H_2O$ systems apart from the melting points of some doubtful hydrates (Heilbron and Bunbury, "Dictionary of Organic Compounds," Eyre and Spottiswoode, 1943).

### Experimental

*Preparation and Purification of Sulphonic Acids.—Benzenesulphonic acid.* The B.D.H. 32% w/v solution was caused to crystallise fractionally at room temperature over phosphoric oxide in a vacuum-desiccator, the main impurity being iron which readily separated as the ferric salt from early fractions. Four-fold crystallisation gave the free acid as colourless deliquescent plates, m. p. 52·5°, whose equivalent by titration with standard barium hydroxide corresponded exactly to the monohydrate. Owing to darkening in bright daylight, the acid was stored in the dark and used only in subdued light.

*Toluene-*o*- and -*p*-sulphonic acids.* Tests showed that the B.D.H. products could be used satisfactorily without further purification. The *p*-acid had m. p. 104·8° and its equivalent corresponded exactly to the monohydrate; the *o*-acid had m. p. 62·1°, with equivalent exactly that of the dihydrate. Repeated crystallisation from water did not give better products or significantly alter certain check solubility results.

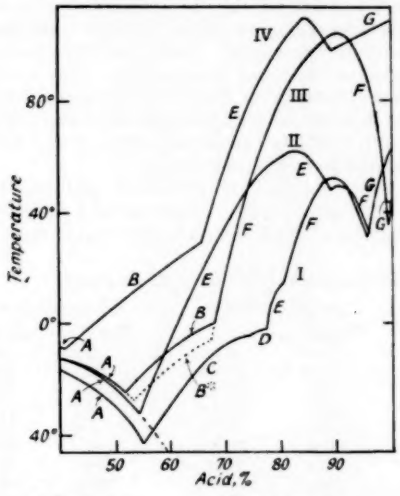
p-*Nitrobenzenesulphonic acid.* Bell's method (*J.*, 1928, 2776) was used and the acid obtained in the pure state *via* the sulphonyl chloride. The latter was crystallised to a sharp m. p. of 77° and then hydrolysed with distilled water, and the resulting solution was evaporated at room temperature over solid potassium hydroxide in a vacuum-desiccator till hydrogen chloride was undetectable. The free acid melted sharply at 110·2° and its equivalent was exactly that of the dihydrate.

p-*Fluorobenzenesulphonic acid.* Fluorobenzene, prepared by Balz and Schiemann's method (*Org. Synth.*, **1939**, **13**, 46), was sulphonated in the cold with 10% oleum. Treatment with barium carbonate followed by phosphorus pentachloride gave the sulphonyl chloride, which was vacuum distilled; it had m. p. 33·7°, and a mixed m. p. with a sample of pure *p*-fluorobenzenesulphonyl chloride 33·9°. The free acid, obtained by hydrolysis in the same manner as the *p*-nitro-acid, melted at 85·8°, and its equivalent corresponded exactly to the monohydrate.

p-*Chlorobenzenesulphonic acid.* This acid was prepared from chlorobenzene in a similar manner to the fluoro-acid. The intermediate sulphonyl chloride melted at 53°, and the free acid at 100·3° (Found : equiv., 210·3. Calc. for $C_6H_5O_3ClS,H_2O$ : equiv., 210·6).

p-*Bromobenzenesulphonic acid.* The B.D.H. sulphonyl chloride, m. p. 75·5°, was hydrolysed and the free acid obtained as for the *p*-nitro-acid; m. p. 90·2° (Found : equiv., 255·4. Calc. for $C_6H_5O_3BrS,H_2O$ : equiv., 255·1).

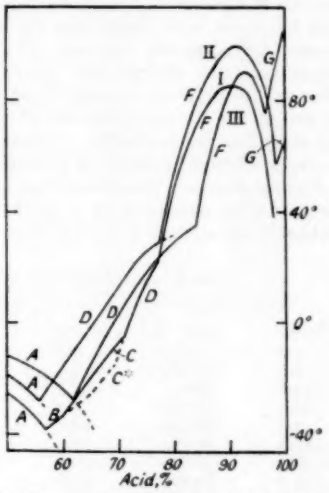
FIG. 1. *Solubility of sulphonic acids.*




*I, Benzene-.* *II, Toluene-o-.*
*III, Toluene-p-.* *IV, p-Nitrobenzene-.*

*Solid phases : A = ice, B = tetrahydrate, C = trihydrate, D = 2·5-hydrate, E = dihydrate, F = monohydrate, G = anhydrous acid, * = metastable form.*

FIG. 2. *Solubility of p-halogen-substituted benzenesulphonic acids.*




*I, Fluoro-. II, Chloro-. III, Bromo-.*
*Solid phases as in Fig. 1.*

All the above sulphonic acids dissolved completely in water and contained no inorganic matter, as shown by negligible residues in sulphated ash tests. For some of the solubility measurements, sulphonic acids containing less water than the above hydrates were required. In the cases of the *o*-Me, *p*-F-, *p*-Cl-, and *p*-Br-acids, dehydration was effected rapidly without decomposition by heating *in vacuo* just above the m. p. of the initial hydrate. The *p*-$NO_2$-acid dihydrate was completely dehydrated in 24 hours at 55° *in vacuo* over phosphoric oxide, benzenesulphonic acid monohydrate required 6 weeks at room temperature, and the *p*-Me acid monohydrate required 6 months at 35°. Higher temperatures for periods of more than a few hours were precluded owing to slight decomposition.

*Solubility Determinations.*—Since these covered a wide range of temperatures and compositions, the synthetic method was used. Sulphonic acid–water mixtures were prepared by weight in small test-tubes which were then sealed and attached to a simple wire framework. The latter was gently rocked, and thermostatic control was provided by manually operated solid carbon dioxide–ethanol, water, or dibutyl phthalate baths according to the temperature required. Supercooling was very pronounced in all cases, and accordingly, mixtures were first completely melted, then cooled and frozen out rapidly by touching the tube with solid carbon dioxide to produce small crystals, and the temperature of complete solution approached from below with careful attention to the rate of heating, after which the contents of the tubes were

titrated with standard barium hydroxide to give the exact composition. The maximum permissible rates were 0·25° per hour below 10°, 1° per hour between 10° and 50°, and 2° per hour above 50°, calibrated thermometers being used throughout. Mixtures were heated above room temperature for the minimum length of time, in which circumstances no decomposition with the formation of sulphuric acid occurred. Where metastable crystals were encountered, the temperature of the initial freezing out was selected so as to produce the desired solid phase. For economy reasons in a few cases, several solution points were determined with the same sample of acid in a bulb of one-inch diameter, to which known amounts of water could be added with a pipette *via* a ground-glass joint and tap. For those parts of the curves between the m. p.s of congruent hydrates and 100% acid, it was frequently more convenient to start with the hydrate in the test-tube or bulb and remove water by evacuation than to prepare mixtures from anhydrous acid and water. For toluene-*o*-sulphonic acid, mixtures in the range 95·7—100% acid could not be induced to crystallise under any conditions, cooling merely producing a glass, and the same applies to the 98—100% range for the fluoro-acid. From Figs. 1 and 2 only the congruent mono- and di-hydrates can be identified directly. The composition of all other hydrates, both stable and metastable, was determined by direct titration: samples were isolated by filtration through sintered-glass filters previously cooled to the appropriate temperature and drying with filter-paper cooled to the same temperature. The number of molecules of water of crystallisation always corresponded very closely to whole numbers or to 2·5, and no results were obtained which were not in harmony both with the solubility curves and with neighbouring hydrates. Results are given in Table 1. In general, Figs. 1 and 2 confirm the expected high stability of monohydrates relatively to higher hydrates.

*Vapour-pressure Determinations.*—The vapour pressures of saturated and unsaturated solutions were measured by a static procedure. The apparatus consisted of a flat-bottomed reaction vessel attached *via* taps and glass sprials to a water reservoir and a wide-bore 15-cm.

TABLE 1. *Solubility of substituted benzenesulphonic acids in water.*

| $C_6H_5{\cdot}SO_3H$ | | *o*-$C_6H_4Me{\cdot}SO_3H$ | | *p*-$C_6H_4Me{\cdot}SO_3H$ | | *p*-$NO_2{\cdot}C_6H_4{\cdot}SO_3H$ | |
|---|---|---|---|---|---|---|---|
| Temp. | Acid, % | Temp. | Acid, % | Temp. | Acid, % | Temp. | Acid, % |
| − 1·5° | 10·42*a* | − 0·7° | 10·26*a* | − 2·0° | 9·67*a* | − 3·0° | 18·88*a* |
| − 4·9 | 21·30*a* | − 3·4 | 19·56*a* | − 4·0 | 19·63*a* | − 5·1 | 30·04*a* |
| −11·5 | 33·9*a* | − 7·2 | 30·42*a* | − 7·5 | 29·45*a* | − 8·5 | 39·74*a* |
| −22·0 | 45·06*a* | −12·8 | 40·38*a* | −13·5 | 41·74*a* | − 8·3 | 41·22*b* |
| −29·8 | 50·23*a* | −25·0 | 51·37*a* | −23·0 | 50·72*a* | − 1·0 | 46·06*b* |
| −40·4 | 54·49*a* | −30·5 | 53·56*a* | −23·5 | 50·97*a* | 10·8 | 53·94*b* |
| −40·8 | 55·91*c* | −33·3 | 54·81*a* ‡ | −24·5 | 51·40*a* ‡ | 16·0 | 56·92*b* |
| −29·0 | 59·96*c* | −37·0 | 56·10*a* ‡ | −25·0 | 51·82*a* ‡ | 26·3 | 63·33*b* |
| −18·5 | 64·18*c* | −39·4 | 57·12*a* ‡ | −25·5 | 52·00*a* ‡ | 36·6 | 66·31*e* |
| −10·0 | 68·87*c* | −44·5 | 59·16*a* ‡ | −25·6 | 52·14*a* ‡ | 56·6 | 69·88*e* |
| − 5·9 | 72·14*c* | −25·0 | 55·69*e* | −18·5 | 54·87*b* | 75·5 | 73·47*e* |
| − 4·7 | 74·44*c* | −13·0 | 58·51*e* | − 5·9 | 62·56*b* | 90·2 | 77·27*e* |
| − 4·0 | 74·87*d* | 0·8 | 61·17*e* | − 3·4 | 64·49*b* | 110·2 | 84·92*e* |
| − 3·3 | 75·82*d* | 16·8 | 64·95*e* | − 2·2 | 65·59*b* | 106·8 | 86·50*e* |
| − 2·5 | 76·40*d* | 31·2 | 68·58*e* | − 1·0 | 66·90*b* | 100·5 † | 90·83*g* |
| − 2·3 | 77·61*d* | 50·0 | 74·78*e* | −27·0 | 53·20*b* * | 105·0 † | 96·13*g* |
| 2·2 | 77·61*e* | 56·0 | 77·07*e* | −26·0 | 54·09*b* * | 110·0 † | 100·0*g* |
| 7·5 | 78·54*e* | 61·2 | 80·90*e* | −16·5 | 59·48*b* * | | |
| 13·7 | 80·04*e* | 62·0 | 83·07*e* | −10·5 | 63·60*b* * | | |
| 15·1 | 80·52*e* | 60·4 | 85·07*e* | − 8·5 | 65·0*b* * | | |
| 21·3 | 81·10*f* | 54·0 | 87·64*e* | − 7·0 | 66·80*b* * | | |
| 31·0 | 82·66*f* | 48·2 | 89·23*e* | − 6·5 | 66·89*f* ‡ | | |
| 39·5 | 84·39*f* | 48·5 | 89·32*f* | − 1·5 | 67·58*f* ‡ | | |
| 49·0 | 87·05*f* | 49·0 | 89·87*f* | 1·5 | 68·06*f* | | |
| 52·4 | 89·60*f* | 49·6 | 91·11*f* | 20·1 | 70·20*f* | | |
| 49·0 | 92·15*f* | 48·6 | 92·05*f* | 38·8 | 72·50*f* | | |
| 39·8 | 94·04*f* | 45·7 | 93·30*f* | 55·2 | 75·23*f* | | |
| 32·6 | 95·35*f* | 38·6 | 94·66*f* | 78·4 | 79·55*f* | | |
| 31·4 | 95·51*g* | 32·5 | 95·65*f* | 89·9 | 82·38*f* | | |
| 42·6 | 96·74*g* | | | 101·1 | 86·98*f* | | |
| 56·0 | 98·42*g* | | | 104·8 | 90·53*f* | | |
| 61·3 | 99·56*g* | | | 102·9 | 92·39*f* | | |
| | | | | 89·1 | 95·37*f* | | |
| | | | | 73·9 | 97·36*f* | | |
| | | | | 45·3 | 99·02*f* | | |
| | | | | 36·5 | 99·73*g* | | |
| | | | | 40·5 | 99·87*g* | | |
| | | | | 42·5 | 99·97*g* | | |

TABLE 1 (*continued*).

| *p*-$C_6H_4F\cdot SO_3H$ | | *p*-$C_6H_4Cl\cdot SO_3H$ | | *p*-$C_6H_4Br\cdot SO_3H$ | |
|---|---|---|---|---|---|
| Temp. | Acid, % | Temp. | Acid, % | Temp. | Acid, % |
| − 4·9° | 19·77*a* | − 3·0° | 19·08*a* | − 1·9° | 14·96*a* |
| −15·0 | 39·81*a* | − 6·5 | 31·96*a* | − 4·5 | 30·21*a* |
| −20·5 | 45·79*a* | −12·5 | 43·76*a* | − 9·0 | 45·07*a* |
| −28·5 | 51·84*a* | −19·5 | 50·04*a* | −12·5 | 50·74*a* |
| −36·4 | 55·63*a* | −23·5 | 53·07*a* | −16·0 | 54·03*a* |
| −38·5 | 56·45*a* | −27·5 | 55·44*a* | −19·5 | 56·94*a* |
| −43·0 | 58·34*a* ‡ | −30·0 | 56·47*a* ‡ | −26·5 | 61·28*a* |
| −38·0 | 57·42*b* | −26·0 | 56·47*d* | −29·5 | 62·46*a* ‡ |
| −35·4 | 58·34*b* | −20·0 | 58·49*d* | −31·5 | 63·39*a* ‡ |
| −34·4 | 59·21*b* | −11·0 | 61·71*d* | −38·0 | 65·21*a* ‡ |
| −32·5 | 60·53*b* ‡ | − 2·2 | 64·71*d* | −21·0 | 63·41*d* |
| −30·5 | 61·38*b* ‡ | 10·6 | 69·34*d* | −10·5 | 65·97*d* |
| −26·4 | 64·54*b* ‡ | 22·9 | 73·60*d* | 0·0 | 68·95*d* |
| −24·0 | 66·25*b* ‡ | 27·6 | 75·98*d* | 12·5 | 72·97*d* |
| −21·4 | 64·54*c* | 30·8 | 78·75*d* ‡ | 19·9 | 75·25*d* |
| −18·5 | 65·25*c* | 41·0 | 78·75*f* | 27·6 | 79·16*d* |
| −13·0 | 67·56*c* | 51·9 | 79·80*f* | 34·6 | 83·49*d* |
| − 7·4 | 69·71*c* | 70·1 | 82·54*f* | 43·8 | 84·78*f* |
| −22·5 | 66·25*c* * | 80·4 | 84·55*f* | 60·2 | 86·72*f* |
| −19·5 | 67·56*c* * | 88·8 | 86·12*f* | 71·2 | 67·92*f* |
| −17·9 | 68·13*c* * | 99·9 | 90·90*f* | 80·3 | 89·78*f* |
| −15·5 | 69·15*d* | 100·2 | 91·94*f* | 86·2 | 91·05*f* |
| − 3·9 | 71·22*d* | 96·7 | 93·78*f* | 90·2 | 92·99*f* |
| 1·0 | 72·02*d* | 89·0 | 95·83*f* | 87·2 | 94·75*f* |
| 10·1 | 74·03*d* | 73·3 | 97·68*f* | 78·5 | 96·18*f* |
| 21·3 | 76·95*d* | 59·8 | 98·31*f* | 76·6 | 96·43*f* |
| 22·1 | 77·14*f* | 59·0 | 99·02*g* | 82·3 | 97·21*g* |
| 35·4 | 78·38*f* | 62·4 | 99·54*g* | 89·6 | 98·00*g* |
| 54·2 | 81·06*f* | | | 93·1 | 98·45*g* |
| 71·2 | 84·33*f* | | | 97·6 | 98·90*g* |
| 80·0 | 86·72*f* | | | | |
| 85·5 | 90·07*f* | | | | |
| 85·2 | 91·64*f* | | | | |
| 81·0 | 94·06*f* | | | | |
| 74·5 | 95·01*f* | | | | |
| 54·3 | 97·24*f* | | | | |
| 41·4 | 98·03*f* | | | | |

*Solid phases*: *a* = ice; *b* = tetrahydrate; *c* = trihydrate; *d* = 2·5 hydrate; *e* = dihydrate; = monohydrate; *g* = anhydrous acid.
* Metastable form. † With decomp. ‡ Metastable.

mercury manometer whose levels were read to ±0·02 mm. with a travelling microscope. The spirals and a glass marble in the reaction vessel allowed gentle agitation of its contents to assist attainment of equilibrium. The system could be evacuated to less than 0·001 mm. of mercury with an oil pump, and was immersed, with the exception of the water reservoir, in a glass-sided water thermostat controlled with electronic relays to better than 0·05°. Below room temperature, a 0° cooling coil was used, and above 80° the water was replaced by dibutyl phthalate. The use of Silicone high-vacuum grease on joints and taps allowed reliable measurements up to 96°. Measurements above this temperature were impracticable owing to slight seepage of dibutyl phthalate into the apparatus, and below 9° because of the very long time to reach equilibrium. Solutions were repeatedly boiled out *in vacuo* to remove dissolved air, compositions being adjusted as desired with de-gassed water from the reservoir. Equilibrium was approached first from below and then from above the equilibrium pressure, and a third time in the case of saturated solutions after a further brief evacuation. The results of these separate experiments, each of which required a maximum of 24 hours, always agreed. Corresponding temperatures and pressures were measured for saturated solutions from beyond the pressure maximum on the mono- or di-hydrate curves, as the case may be, to below the transition to the next highest hydrate.

Measurements at 50° on unsaturated solutions, whose compositions were determined by titration, covered a short composition range up to and including saturation. In no case was more than a minute trace of free sulphuric acid detected in the solutions at the end of an experiment. Previous tests using distilled water gave vapour pressures agreeing within the limits of the microscope with values given in International Critical Tables, **3**, 210, thus confirming

the accuracy of the procedure. In Tables 2 and 3 all pressures are given in mm. of mercury corrected to 0°.

TABLE 2. *Vapour pressure (mm. Hg) of saturated aqueous solutions of substituted benzenesulphonic acids.*

| $C_6H_5\cdot SO_3H$ | | $o$-$C_6H_4Me\cdot SO_3H$ | | $p$-$NO_2\cdot C_6H_4\cdot SO_3H$ | | $p$-$C_6H_4F\cdot SO_3H$ | | $p$-$C_6H_4Cl\cdot SO_3H$ | | $p$-$C_6H_4Br\cdot SO_3H$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Temp. | Mm. | Temp. | Mm. | Temp. | Mm. | Temp. | Mm. | Temp. | Mm. | Temp. | Mm. |
| 9·0° | 1·24*e* | 25·0° | 12·55*e* | 21·0° | 12·93*b* | 12·0° | 3·21*d* | 22·0° | 6·62*d* | 20·1° | 4·74*d* |
| 13·0 | 1·65*e* | 35·0 | 20·32*e* | 23·0 | 14·25*b* | 14·0 | 3·49*d* | 24·0 | 7·43*d* | 26·0 | 6·63*d* |
| 15·0 | 1·86*e* | 45·0 | 30·09*e* | 25·0 | 15·60*b* | 16·0 | 3·84*d* | 26·0 | 8·26*d* | 28·0 | 7·39*d* |
| 16·0 | 1·95*e* | 55·0 | 39·85*e* | 27·0 | 16·87*b* | 18·0 | 4·16*d* | 28·0 | 9·15*d* | 30·0 | 8·20*d* |
| 17·1 | 2·02*e* | 57·0 | 40·66*e* | 28·0 | 17·55*b* | 20·0 | 4·54*d* | 29·0 | 9·59*d* | 32·0 | 9·02*d* |
| 18·0 | 2·05*e* | 58·0 | 41·03*e* | 29·05 | 18·44*e* | 22·0 | 4·98*f* | 30·0 | 10·13*f* | 35·0 | 10·51*f* |
| 18·95 | 2·12*f* | 58·5 | 41·05*e* | 31·0 | 20·41*e* | 24·0 | 5·62*f* | 32·0 | 11·28*f* | 38·0 | 12·10*f* |
| 23·05 | 2·69*f* | 59·0 | 41·03*e* | 33·0 | 22·66*e* | 28·0 | 6·91*f* | 35·0 | 13·09*f* | 40·0 | 13·24*f* |
| 31·0 | 3·94*f* | 60·0 | 40·90*e* | 40·0 | 31·75*e* | 35·0 | 9·80*f* | 39·8 | 16·22*f* | 50·0 | 20·27*f* |
| 39·0 | 5·14*f* | | | 50·0 | 49·58*e* | 55·0 | 22·65*f* | 60·0 | 38·19*f* | 60·0 | 29·32*f* |
| 43·0 | 5·69*f* | | | 65·0 | 91·64*e* | 70·0 | 38·06*f* | 80·1 | 73·30*f* | 70·0 | 39·60*f* |
| 46·0 | 5·90*f* | | | 79·0 | 143·4*e* | 75·0 | 42·01*f* | 88·9 | 85·59*f* | 80·1 | 52·25*f* |
| 48·0 | 5·98*f* | | | | | 77·1 | 43·03*f* | 91·0 | 47·48*f* | 83·9 | 53·89*f* |
| 50·0 | 5·19*f* | | | | | 79·0 | 43·72*f* | 93·4 | 88·41*f* | 84·4 | 53·91*f* |
| | | | | | | 80·0 | 43·74*f* | 94·3 | 88·24*f* | 84·95 | 53·89*f* |
| | | | | | | 83·1 | 41·37*f* | 96·2 | 87·00*f* | 85·8 | 53·35*f* |

*Solid phases*: As in Table 1.

TABLE 3. *Vapour pressure (mm. Hg) of unsaturated aqueous solutions of substituted benzenesulphonic acids at 50·0°.**

| $C_6H_5\cdot SO_3H$ | | $o$-$C_6H_4Me\cdot SO_3H$ | | $p$-$C_6H_4Me\cdot SO_3H$ | | $p$-$NO_2\cdot C_6H_4\cdot SO_3H$ | |
|---|---|---|---|---|---|---|---|
| Mm. | *m* | Mm. | *m* | Mm. | *m* | Mm. | *m* |
| 14·23 | 27·23 | 42·89 | 14·38 | 46·44 | 13·03 | 61·02 | 8·312 |
| 11·53 | 30·18 | 40·89 | 15·02 | 44·92 | 13·60 | 57·50 | 8·991 |
| 9·11 | 33·36 | 39·99 | 15·27 | 43·86 | 14·01 | 54·93 | 9·543 |
| 8·34 | 34·18 | 38·48 | 15·80 | 41·44 | 14·69 | 53·99 | 9·831 |
| 7·15 | 38·07 | 37·87 | 16·14 | 39·37 | 15·45 | 53·56 | 9·959 |
| 6·37 | 40·16 | 36·56 | 16·85 | 37·38 | 16·12 | 52·55 | 10·22 |
| 6·18 | 41·78 | 36·11 | 17·11 | 36·87 | 16·29 | 50·82 | 10·58 |
| 5·88 | 42·24 | 35·86 | 17·22 † | 35·90 | 16·60 | 50·43 | 10·66 |
| 5·91 | 43·65 † | | | 35·33 | 16·83 † | 49·66 | 10·90 † |

| $p$-$C_6H_4F\cdot SO_3H$ | | $p$-$C_6H_4Cl\cdot SO_3H$ | | $p$-$C_6H_4Br\cdot SO_3H$ | |
|---|---|---|---|---|---|
| Mm. | *m* | Mm. | *m* | Mm. | *m* |
| 24·96 | 20·03 | 33·23 | 16·54 | 24·90 | 20·61 |
| 23·48 | 20·55 | 31·56 | 17·48 | 22·25 | 22·21 |
| 22·61 | 20·88 | 30·09 | 18·06 | 21·85 | 22·58 |
| 22·04 | 21·30 | 29·23 | 18·45 | 21·41 | 23·09 |
| 21·51 | 21·72 | 28·21 | 18·96 | 20·91 | 23·64 |
| 20·50 | 22·21 | 27·55 | 19·27 | 20·21 | 24·73 † |
| 19·42 | 23·07 | 26·63 | 19·74 | | |
| 18·63 | 23·45 † | 25·57 | 20·24 † | | |

* *m* = G.-mole of solute per 1000 g. of water. † Saturated.

Solid phases at saturation: monohydrate for benzene, *p*-$CH_3$, *p*-F, *p*-Cl, *p*-Br; dihydrate for *o*-$CH_3$ and *p*-$NO_2$.

*Dissociation Pressure of Monohydrates.*—The same apparatus was used as for solution vapour pressures. Monohydrate was crystallised *in vacuo* in the reaction vessel, and approximately half the water of crystallisation removed by prolonged evacuation before allowing the system to come to equilibrium. The times required ran into months and frequently at the end of an experiment traces of free sulphuric acid could be detected. Reliable results were obtained in two cases only.

For benzenesulphonic acid monohydrate, the dissociation pressures were:

| Temp. | 24·00° | 24·90° | 25·90° | 26·85° | 27·90° | 29·10° | 29·85° |
|---|---|---|---|---|---|---|---|
| *P*, mm. Hg | 0·096 | 0·105 | 0·120 | 0·140 | 0·155 | 0·175 | 0·200 |

Each of these pressures was the average of ten readings. They do not deviate by more than about 3% from the equation $\log_{10} P = -4922/T + 15{\cdot}54$, the heat of dissociation being 22·5 kcal./mole. Results (average of 10 readings) for the *p*-bromo-acid were:

| Temp. | 40·0° | 50·0° | 54·8° |
|---|---|---|---|
| *P*, mm. Hg | 0·75 | 1·54 | 2·11 |

Agreement is better than 1% with the equation $\log_{10} P = -3087/T + 9{\cdot}743$, giving a heat of dissociation of 14·1 kcal./mole. Attempts to approach equilibrium by adding water vapour to

a pressure initially greater than the dissociation pressure were vitiated by rapid adsorption on the anhydrous acid phase. For the *p*-bromo-acid, adsorption equilibrium pressures (average of 10 readings) were :

| Temp. | 41° | 45° | 50° | 55·1° | 60·9° | 65·2° | 67·0° | 69·9° | 72·04° |
|---|---|---|---|---|---|---|---|---|---|
| *P*, mm. Hg | 0·26 | 0·34 | 0·54 | 0·86 | 1·21 | 1·84 | 2·11 | 2·44 | 2·84 |

They agree closely with the equation $\log_{10} P = -3663/T + 11{\cdot}07$. The pressure remained at the low adsorption value for several hours before very slowly rising to the true dissociation value.

*Pressure Maxima on the Three-phase Curves.*—Below are given the observed maxima and those calculated from Dingemans's equation $1/T_{max.} = 1/T_f + 0{\cdot}00021$, where $T_f$ is the m. p. in °K of the stable solid phase. Dingemans has shown (*loc. cit.*) that this equation applies with remarkable success to inorganic compounds where $T_{max.}$ is 20—30° less than $T$. In the present work $T_{max.}$ is close to $T_f$, and failure is marked :

| Acid | $T_f$, °c | $T_{max.}$, obs., °c | $T_{max.}$, calc., °c | Acid | $T_f$, °c | $T_{max.}$, obs., °c | $T_{max.}$, calc., °c |
|---|---|---|---|---|---|---|---|
| † $C_6H_5$· ..... | 52·5 | 48·0 | 31·7 | † *p*-Cl·$C_6H_4$· | 100·4 | 93·4 | 73·4 |
| * *o*-$CH_3$·$C_6H_4$· | 62·2 | 58·5 | 40·2 | † *p*-Br·$C_6H_4$· | 90·3 | 84·4 | 64·7 |
| † *p*-F·$C_6H_4$· ... | 85·7 | 80·8 | 60·3 | | | | |

Stable solid phases : * dihydrate, † monohydrate.

*Heats of Solution at 50°.*—These were calculated from Williamson's equation 49 (*loc. cit.*, Appendix). The data in Table 1 were recalculated in terms of molalities, and $(dm/dT)_{sat.}$ evaluated graphically. The term $\left(\frac{\partial \ln \alpha}{\partial m}\right)_T = \frac{1}{P}\left(\frac{\partial P}{\partial m}\right)_T$, where $\alpha$ and $P$ are respectively the activity and vapour pressure of the solvent, was determined graphically at saturation from the data in Table 3. The heat of solution at saturation is then given by

$$\left(x - \frac{55{\cdot}51}{m}\right) RT^2 \left(\frac{dm}{dT}\right)_{sat.} \frac{1}{P}\left(\frac{\partial P}{\partial m}\right)_T$$

where $x$ is the number of molecules of water of crystallisation in the stable solid phase. Results were as follows, in which estimated errors allow only for errors in drawing tangents :

| R in $C_6H_4R{\cdot}SO_3H$ | Heat of soln., kcal./mole | R in $C_6H_4R{\cdot}SO_3H$ | Heat of soln., kcal./mole | R in $C_6H_4R{\cdot}SO_3H$ | Heat of soln., kcal./mole |
|---|---|---|---|---|---|
| H, $+H_2O$ ..... | 1·8 ± 0·2 | *p*-$NO_2$, $+2H_2O$ ... | 4·3 ± 0·2 | *p*-Cl, $+H_2O$ ..... | 3·8 ± 0·2 |
| *o*-Me, $+2H_2O$ ... | 5·1 ± 0·3 | *p*-F, $+H_2O$ ..... | 4·3 ± 0·2 | *p*-Br, $+H_2O$ ..... | 1·7 ± 0·1 |
| *p*-Me, $+H_2O$ ... | 6·2 ± 0·2 | | | | |

*Heats of Dissociation of Hydrates Other than Monohydrates.*—Full details for the approximate calculation of dissociation pressures from a combination of stable and metastable saturated solution vapour pressures are given by Pedersen (*loc. cit.*). For a selection of hydrates, the calculated pressures and heats of dissociation are given in Table 4. In view of the extrapolation

TABLE 4. *Heats of dissociation.*

| Acid | Hydrate equilibrium | Calculations after Pedersen : Temp. | Dissocn. press., mm. Hg | Heat of dissocn., kcal./mole | Heat of dissocn., from $\Delta H$ values, kcal./mole |
|---|---|---|---|---|---|
| $C_6H_5{\cdot}SO_3H$ ..... | $2H_2O \rightleftharpoons H_2O$ | 14·0° | 1·47 | 10·5 | 10·5 |
| | | 9·0 | 1·05 | | |
| *p*-$NO_2{\cdot}C_6H_4{\cdot}SO_3H$ ... | $4H_2O \rightleftharpoons 2H_2O$ | 27·0 | 15·78 | 14·7 | 14·0 |
| | | 22·0 | 10·43 | | |
| *p*-$C_6H_4F{\cdot}SO_3H$ ..... | $2{\cdot}5H_2O \rightleftharpoons H_2O$ | 20·0 | 4·43 | 15·4 | 11·4 |
| | | 15·0 | 3·21 | | |
| *p*-$C_6H_4Cl{\cdot}SO_3H$ ..... | $2{\cdot}5H_2O \rightleftharpoons H_2O$ | 27·0 | 8·70 | 12·4 | 9·4 |
| | | 22·0 | 6·13 | | |
| *p*-$C_6H_4Br{\cdot}SO_3H$ ..... | $2{\cdot}5H_2O \rightleftharpoons H_2O$ | 30·0 | 8·07 | 12·1 | 9·1 |
| | | 25·0 | 5·73 | | |

of the data in Table 2 required to give vapour pressures of metastable solutions, and the small differences between these and the pressures of stable solutions, the heat of dissociation values are probably not accurate to better than ±1 kcal.

Dingemans's approximate treatment (*loc. cit.*) of heats of transition of polymorphs of anhydrous compounds can be extended to hydrates to give heats of dissociation as follows. In the case of 2·5 hydrate ⇌ monohydrate equilibria, let 1 mole of saturated solution at the

transition point contain $x$ moles of anhydrous acid and $(1 - x)$ moles of water. On isothermal evaporation of the solution at a temperature infinitesimally greater than the transition point,

$\Delta H_1 + [1/(1 - 2x)]$ mole of sat. soln. ⟶ 1 mole of water vapour + $[x/(1 - 2x)]$ mole of monohydrate

where $\Delta H_1$ is the change of heat content accompanying the formation of the saturated solution from solid monohydrate and solvent vapour and may be evaluated from the gradient of the three-phase curve (Glasstone, " Thermodynamics for Chemists," Van Nostrand, 1947, p. 241). Similarly, for crystallisation of the 2·5 hydrate at a temperature infinitesimally lower than the transition point,

$\Delta H_{2\cdot5} + [1/(1 - 3{\cdot}5x)]$ mole of sat. soln. ⟶ 1 mole of water vapour + $[x/(1 - 3{\cdot}5x)]$ mole of 2·5 hydrate.

Rearrangement and subtraction of these two equations gives

$2/3\{[(1 - 2x)/x]\ \Delta H_1 - [(1 - 3{\cdot}5x)/x]\ \Delta H_{2\cdot5}\} + 2/3$ mole of 2·5 hydrate ⟶ 1 mole of water vapour + 2/3 mole of monohydrate

in which the first term is the heat of dissociation of the 2·5 hydrate at the transition point. The dissociation of benzenesulphonic acid dihydrate and of the *p*-nitro-acid tetrahydrate may be treated similarly. In certain cases, to determine the $\Delta H$ value the plot of $\log_{10} P$ against $1/T$ results in two very similar gradients above and below the transition point. This introduces no major error, however, since both $\Delta H$ quantities are multiplied by composition factors, and the respective products are appreciably different. The data in Tables 1 and 2 being used, the values in Table 4 were obtained for which an accuracy of not more than ±1 kcal. is claimed. The agreement between these and Pedersen's values is as good as can be expected considering the approximate nature of the treatments. In the case of monohydrates of benzene- and *p*-bromobenzene-sulphonic acids, dissociation pressures are much smaller and heats of dissociation greater than for higher hydrates, thus confirming the higher relative stability of their monohydrates evident from Figs. 1 and 2.

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## 617. *Deoxypentosenucleic Acids. Part IV.* The Properties and Composition of the Deoxypentosenucleic Acids from Certain Animal, Plant, and Bacterial Sources.*

By S. G. LALAND, W. G. OVEREND, and M. WEBB.

A number of deoxypentosenucleic acids from certain animal, plant, and bacterial sources, have been analysed in various ways. An analytical study has also been made of the compositions of the enzyme-resistant " cores " obtained by the action of deoxyribonuclease on the nucleic acid preparations.

As part of an investigation of the composition and specificity of deoxypentosenucleic acids, the properties of a number of nucleic acid preparations have been studied by the usual well-established methods. The purine and pyrimidine contents of the deoxypentosenucleic acids and of their enzyme-resistant " cores " (obtained by the action of deoxyribonuclease) have been determined by the chromatographic technique.

Notwithstanding the fact that such analyses have already been carried out in a number of laboratories (cf., *e.g.*, Vischer and Chargaff *J. Biol. Chem.*, 1948, **176**, 715; Chargaff, Vischer, Doniger, Green, and Misani, *ibid.*, 1949, **177**, 405; Magasanik, Vischer, Doniger, Elson, and Chargaff, *ibid.*, 1950, **186**, 37; Wyatt, *Biochem. J.*, 1951, **48**, 581; 1951, **49**, 144;

* Part III, *J.*, 1952, 303.

Daly, Allfrey, and Mirsky, *J. Gen. Physiol.*, 1950, **33**, 497), the present results are presented here since it is considered that, with such ill-defined materials as deoxypentosenucleic acids, the full significance of the results can only be determined by comparison of the data accumulated in independent investigations.

Representative of the nucleic acids from animal tissues, deoxypentosenucleic acid preparations were isolated (in the frozen-dried state) from calf thymus (preparations $T_1$ and $T_2$) and beef spleen by essentially the methods of Mirsky and Pollister (*Proc. Nat. Acad. Sci. Wash.*, 1942, **144**, 383; *J. Gen. Physiol.*, 1946, **30**, 117) and Peterman and Lamb (*J. Biol. Chem.*, 1948, **176**, 685; but cf. Webb, *Nature*, 1952, **169**, 417). Additional preparations were derived from herring testis (preparations $H_1$ and $H_2$, isolated according to the method of Mirsky and Pollister, *loc. cit.*), and a transplantable mouse sarcoma, originally induced by the intramuscular injection of 1 : 2 : 5 : 6-dibenzanthracene (the authors are indebted to Dr. D. L. Woodhouse for the deoxypentosenucleic acid preparation from this source). Wheat-germ deoxypentosenucleic acid (isolated as described by Laland, Overend, and Webb, *Acta Chem. Scand.*, 1950, **4**, 885) was examined as a typical plant nucleic acid. Bacterial deoxypentosenucleic acids were represented by samples isolated from *Mycobacterium tuberculosis* (human strain) and *Mycobact. phlei* by a method (unpublished) developed in collaboration with Dr. A. S. Jones.

These preparations (with the exception of those derived from wheat germ, mouse-sarcoma tissue, and *Mycobact. phlei*) contained *ca.* 1% of pentosenucleic acid (Table 1) as determined by Euler and Hahn's method (*Svensk Kem. Tidskr.*, 1946, **10**, 251). Colour tests for proteins were negative.

Certain properties and characteristics of the preparations are recorded in Table 1. The significance of some of these results must be briefly discussed.

*Nitrogen : Phosphorus Ratio.*—Values (1·62) obtained for the deoxypentosenucleic acid preparations from calf thymus agree with those quoted by Gulland *et al.* (*J.*, 1947, 1129) and by Chargaff *et al.* (*J. Biol. Chem.* 1949, **177**, 429). Other values, with the exception of that found for the deoxypentosenucleic acid of *Mycobact. tuberculosis*, do not differ appreciably from each other. The low value (1·32) found for the N : P ratio of the *Mycobact. tuberculosis* deoxypentosenucleic acid is in agreement with the value (1·39) reported by Brown and Johnson (*J. Amer. Chem. Soc.*, 1923, **45**, 1823) for tuberculinic acid, but differs from that reported by Chargaff *et al.* (*loc. cit.*, p. 429) for a preparation of the deoxypentosenucleic acid of avian tubercle bacilli (*i.e.*, 1·80).

*Dische and Feulgen Values.*—The Dische values obtained with the deoxypentosenucleic acids of calf thymus, beef spleen, and herring testis are all of similar magnitude. This is possibly correlated with the fact that the purine contents of these nucleic acids are similar (Table 1, cf. Mirsky, *Adv. Enzymol.*, 1943, **3**, 1). The Dische value given by the wheat-germ deoxypentosenucleic acid is slightly lower than those of the above nucleic acids, in agreement with the lower purine content of the preparation. However with the deoxypentosenucleic acid from *Mycobact. phlei* no relation is apparent between the Dische value and the purine content of the preparation. This discrepancy may be due to the fact that purines are not so readily liberated by acid from bacterial deoxypentosenucleic acids as from other deoxypentosenucleic acids (Vischer, Zamenhof, and Chargaff, *J. Biol. Chem.*, 1949, **177**, 429).

The Feulgen values recorded do not appear to show any significant differences.

*Chromatographic Analysis of the Deoxypentosenucleic Acids.*—Samples of the deoxypentosenucleic acids were hydrolysed at 175° for 50 minutes with formic acid, and the free bases then separated by paper chromatography. The chromatograms were run with a solvent mixture of *n*-butanol, 0·5N-hydrochloric acid and, 2-methoxyethanol (30 : 17 : 10; v/v/v). The bases were located on the chromatograms by the ultra-violet photographic technique (Markham and Smith, *Biochem. J.*, 1949, **45**, 295; 1950, **46**, 509). Control experiments with standard mixtures of authentic purines and pyrimidines showed that the method had an error not greater than $\pm 5\%$ with adenine, guanine, cytosine, and thymine, and $\pm 10\%$ with 5-methylcytosine.

Comparison of the results of these determinations with previously published values (Table 2) shows that the present results are in closest agreement with those of Wyatt

TABLE 1.

| Source of deoxypentose nucleic acid | Nitrogen content (%) [1] | Phosphorus content (%) [2] | N/P | Pentose nucleic acid content (%) [3] | Ultra-violet absorption [4] $\varepsilon_{(P)259}$ | $\varepsilon_{(P)230}$ | $\varepsilon_{(P)280}$ | $\varepsilon_{(P)257}$ | Relative viscosity, $\eta_r$ [5] | Purine-N / Pyrimidine-N [6] | Mols. of purines per 4 atoms of phosphorus | Dische value [7] | Feulgen value [8] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Calf thymus ($T_1$) ..... | 12·8 | 7·90 | 1·62 | 1·3 | 7700 | 3350 | 4450 | — | 3·60 | 1·60 | 1·76 | 2050 | 810 |
| Calf thymus ($T_2$) ..... | 12·9 | 7·95 | 1·62 | 0·8 | 7900 | 3600 | 4600 | — | 4·80 | — | 1·78 | 2200 | 800 |
| Herring testis ($H_1$) ..... | 13·4 | 8·35 | 1·61 | 0·4 | 7500 | 3070 | 4100 | — | 1·85 | 1·60 | 1·71 | 2030 | 790 |
| Herring testis ($H_2$) ..... | 13·4 | 8·50 | 1·58 | 0·4 | 7600 | 3170 | 4250 | — | 1·80 | — | 1·70 | 2050 | 780 |
| Beef spleen ..... | 12·0 | 7·75 | 1·55 | 1·8 | 6750 | 3200 | 3900 | — | 1·41 | — | 1·75 | 1950 | 740 |
| Mouse sarcoma ..... | 12·7 | 8·10 | 1·57 | 15·7 | 7800 | 3550 | 4350 | — | 1·67 | — | 1·65 | 1780 | 750 |
| Wheat germ ..... | 13·7 | 8·65 | 1·59 | 4·8 | 7300 | 3015 | 3900 | — | 1·33 | 1·57 | 1·57 | 1860 | 790 |
| *Mycobact. tuberculosis* ... | 10·8 | 8·20 | 1·32 | 1·3 | — | 3500 | 4430 | 6550 | — | — | 1·28 | 1370 | — |
| *Mycobact. phlei* ..... | 11·7 | 7·80 | 1·50 | 5·8 | — | 3200 | 3700 | 6750 | 1·90 | — | 1·57 | 1230 | 750 |

[1] Estimated by the micro-Kjeldahl method (Markham, *Biochem. J.*, 1942, **36**, 790).
[2] Determined by Allen's method (*Biochem. J.*, 1940, **34**, 858) as modified by Jones, Lee, and Peacocke (*J.*, 1951, 623).
[3] Method of Euler and Hahn (*Svensk Kem. Tidskr.*, 1946, **10**, 251).
[4] Determined on nucleic acid solutions (containing *ca.* 0·06 mg./c.c.) with a Unicam spectrophotometer. The values reported are atomic extinction coefficients with respect to the phosphorus content of the nucleic acid (cf. Chargaff and Zamenhof, *J. Biol. Chem.*, 1948, **173**, 327); all preparations exhibited maximum absorption at 259 mμ with the exception of the two microbial deoxyribonucleic acids which had maximum absorption at 257 mμ.
[5] The values recorded were obtained in Ostwald viscometers at 25° with 0·05% aqueous solutions of the nucleic acids (previously dried at 80°/20 mm. over phosphoric oxide).
[6] Method of Gulland *et al.* (*J.*, 1947, 1129) (cf. the differences in the values determined by this method and the chromatographic technique, Table 2).
[7] The deoxypentosenucleic acid solution (0·5 c.c. of a solution containing 6 mg./10 c.c.) was heated with water (1·5 c.c.) and the Dische reagent (4 c.c.) (cf. Stacey *et al.*, *J.*, 1949, 1222) for 15 mins. at 100°. The intensity of the colour developed was measured in the Spekker absorptiometer with Ilford filters No. 606. Values recorded are atomic extinction coefficients with respect to the phosphorus contents of the nucleic acids.
[8] Values are calculated on same basis as the Dische values. Colour developed according to Widström's procedure (*Biochem. Z.*, 1928, **199**, 298) with Tobie's modification (*Ind. Eng. Chem., Anal.*, 1942, **14**, 405) of Schiff's reagent.

TABLE 2.

| Source of deoxyribonucleic acid | Investigator | Molar proportions of bases.* A/C | G/C | T/C | G/T | Mols. of bases/4 atoms of phosphorus A | G | C | T | Mc | Total | Ratio mols. purines/ mols. pyrimidines | Purine-N / Pyrimidine-N | N/P | Nitrogen recovery (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Calf thymus | This paper (prepn. $T_1$) | 1·30 | 1·01 | 1·31 | 0·77 | 0·99 | 0·77 | 0·76 | 1·00 | 0·07 | 3·59 | 0·96 | 1·98 | 1·59 | 96·5 |
| | ,, (prepn. $T_2$) | 1·31 | 0·97 | 1·29 | 0·75 | 1·02 | 0·76 | 0·78 | 1·01 | 0·07 | 3·64 | 0·96 | 1·96 | 1·61 | 94·0 |
| | Wyatt [1] | 1·29 | 0·98 | 1·30 | 0·75 | 0·98 | 0·75 | 0·76 | 0·99 | 0·05 | | | | | |
| | Chargaff *et al.*[2] | 1·47 | 1·16 | 1·31 | 0·88 | 1·12 | 0·88 | 0·76 | 1·00 | — | | | | | |
| | Mirsky *et al.*[3] | 1·40 | 1·19 | 1·42 | 0·84 | 1·10 | 0·94 | 0·79 | 1·12 | — | | | | | |
| Beef spleen | This paper | 1·38 | 1·01 | 1·37 | 0·74 | 1·01 | 0·74 | 0·73 | 1·00 | 0·05 | 3·53 | 0·99 | 2·03 | 1·54 | 96·5 |
| | Wyatt [1] | 1·34 | 1·00 | 1·34 | 0·75 | 1·02 | 0·76 | 0·76 | 1·02 | 0·05 | | | | | |
| | Chargaff *et al.*[2] | 1·51 | 1·19 | 1·41 | 0·84 | 1·04 | 0·82 | 0·69 | 0·97 | — | | | | | |
| Herring testis | This paper (prepn. $H_1$) | 1·29 | 0·90 | 1·31 | 0·70 | 1·00 | 0·71 | 0·82 | 1·05 | 0·10 | 3·68 | 0·89 | 1·74 | 1·56 | 97·5 |
| | ,, (prepn. $H_2$) | 1·30 | 0·91 | 1·31 | 0·69 | 1·00 | 0·70 | 0·77 | 1·01 | 0·10 | 3·58 | 0·91 | 1·84 | 1·57 | 94·5 |
| | Wyatt [1] | 1·31 | 1·06 | 1·31 | 0·81 | 1·00 | 0·81 | 0·76 | 1·00 | 0·07 | | | | | |
| Wheat germ | This paper | 1·38 | 1·03 | 1·40 | 0·74 | 0·90 | 0·67 | 0·65 | 0·91 | 0·19 | 3·32 | 0·88 | 1·80 | 1·55 | 89·5 |
| | Wyatt [1] | 1·51 | 1·34 | 1·54 | 0·86 | 0·89 | 0·79 | 0·59 | 0·91 | 0·19 | | | | | |
| | Brawerman and Chargaff [4] | 1·62 | 1·34 | 1·61 | 0·83 | 1·05 | 0·87 | 0·65 | 1·04 | 0·23 | | | | | |
| | Mirsky *et al.*[3] | 1·67 | 1·42 | 1·64 | 0·87 | 1·10 | 0·94 | 0·66 | 1·08 | — | | | | | |
| *Mycobact. tuberculosis*, human strain | This paper | 0·55 | 0·81 | 0·50 | 1·62 | 0·52 | 0·76 | 0·94 | 0·47 | — | 2·69 | 0·91 | 1·70 | 1·27 | 94·0 |
| | Wyatt [5] | 0·54 | 0·85 | 0·60 | 1·43 | | | | | | | | | | |
| *Mycobact. tuberculosis*, avian strain | Chargaff *et al.*[2] | 0·47 | 1·10 | 0·40 | 2·75 | 0·48 | 1·12 | 1·02 | 0·41 | — | | | | | |
| *Mycobact. phlei* | This paper | 0·52 | 0·91 | 0·45 | 2·04 | 0·57 | 1·00 | 1·10 | 0·49 | — | 3·16 | 0·98 | 1·85 | 1·50 | 92·5 |
| Mouse sarcoma | This paper | 1·30 | 0·96 | 1·41 | 0·85 | 0·95 | 0·70 | 0·73 | 0·82 | — | 3·20 | 1·05 | 2·16 | 1·57 | 87·5 |

* A = Adenine, G = guanine, C = cytosine, T = thymine, Mc = 5-methylcytosine, N/P = nitrogen/phosphorus ratio of the nucleic acid hydrolysate placed on chromatogram.

[1] Wyatt, *Biochem. J.*, 1951, **48**, 584.
[2] Chargaff *et al.*, *J. Biol. Chem.*, 1949, **177**, 429; 1949, **177**, 405; 1950, **187**, 1.
[3] Daly, Mirsky, and Allfrey, *J. Gen. Physiol.*, 1950, **33**, 497.
[4] Brawerman and Chargaff, *J. Amer. Chem. Soc.*, 1951, **73**, 4052.
[5] Wyatt, *Biochem. J.*, 1951, **49**, 144.

Chargaff *et al.* have published results obtained with several preparations of calf-thymus deoxypentosenucleic acid (which exhibit some variation in composition) and with two preparations of beef-spleen deoxypentosenucleic acid; Mirsky *et al.* have recorded the results on three preparations of calf-thymus deoxypentosenucleic acid.

(*loc. cit.*). Table 2 shows, however, that the nitrogen recoveries for all the deoxpentosenucleic acids examined were low and ranged from 87·5% (mouse-sarcoma deoxypentosenucleic acid) to 97·5% (beef-spleen deoxypentosenucleic acid). Low nitrogen-recovery figures are also characteristic of results obtained by other workers (cf. Chargaff *et al.*, *loc. cit.*, p. 405; Daly *et al.*, Wyatt, *locc. cit.*) and may be due to such factors as, *e.g.*, incomplete hydrolysis, destruction of the bases during hydrolysis, incomplete recovery of the bases from the chromatograms, and the presence of nitrogenous contaminants (*e.g.*, protein) in the nucleic acid preparations. Moreover, small amounts of nitrogenous components in the deoxypentosenucleic acids may have so far escaped detection. In the present experiments it is considered unlikely that incomplete hydrolysis is the cause of the low nitrogen-recovery figures, since longer times of hydrolysis did not alter the results obtained. Destruction of bases during hydrolysis, and incomplete recovery of the bases from the chromatograms, are also considered unlikely, since experiments with standard mixtures gave satisfactory results within the limit of experimental error.

The content of 5-methylcytosine found for the wheat-germ deoxypentosenucleic acid (29·2; expressed as a molar percentage of the cytosine content) agrees well with the value (33) reported by Wyatt (*loc. cit.*) for a similar preparation. Values (expressed as above) for the 5-methylcytosine content of the deoxypentosenucleic acids of calf thymus, herring testis, and beef spleen are somewhat higher than those reported by Wyatt (*loc. cit.*). In agreement with the results obtained by Chargaff *et al.* (*J. Biol. Chem.*, 1949, **177**, 429) with the deoxypentosenucleic acid of avian tubercle bacilli, and of Wyatt (*loc. cit.*), the deoxypentosenucleic acids isolated from *Mycobact. tuberculosis* (human strain) and *Mycobact. phlei* contained no detectable amount of 5-methycytosine.

The results (Table 2) also show that the deoxypentosenucleic acid isolated from wheat germ is similar in composition to the animal nucleic acids, but contains slightly less of all four bases per four atoms of phosphorus, and is characterised by a much higher 5-methylcytosine content. The considerable differences in composition between the bacterial nucleic acids and the other nucleic acids examined, are also apparent from the Table.

*Enzymic Degradation of the Deoxypentosenucleic Acids and Isolation of the Enzyme-resistant Residues* ("*Cores*") (cf. Zamenhof and Chargaff, *J. Biol. Chem.*, 1949, **178**, 531; 1950, **187**, 1; Overend and Webb, *J.*, 1950, 2746).

Solutions of the deoxypentosenucleic acids at pH 7·1 were incubated for 15 hours at 37° with deoxyribonuclease under the conditions defined in the Experimental section, and then dialysed against frequent changes of distilled water until free from dialysable products. The non-diffusible residues were evaporated in the frozen state. Fractions of the residues (or "cores") were hydrolysed and analysed by the chromatographic technique as described in the preceding section. The results obtained are summarised in Table 3. The figures recorded for adenine, guanine, cytosine, and thymine are the means of five estimations, and those for 5-methylcytosine the means of two estimations.

The non-diffusible "cores" obtained after one treatment with the enzyme and subsequent dialysis comprised a relatively large residue, which in most cases amounted to at least 50% of the original nucleic acid. A second treatment of certain of the "cores" with deoxyribonuclease followed by dialysis of the enzymic degradation products afforded further non-diffusible residues, which amounted to approximately 20% of the original nucleic acid. These "cores" differ from those obtained by Zamenhof and Chargaff (*loc. cit.*) who, in some cases, obtained from calf-thymus deoxypentosenucleic acid after one treatment with deoxyribonuclease a non-diffusible "core" comprising only 7% of the original nucleic acid. These differences may be due to the fact that in the present experiments dialysis of the enzyme degradation products was carried out at room temperature against distilled water changed at noted intervals of time, and not against buffer solutions at 37°. Repeated changes of distilled water serve to remove metallic ions, which are known to favour the dialysis of polynucleotides (Lehmann-Echternacht, *Z. physiol. Chem.*, 1941, **269**, 169).

Some comparison of the present results with those reported by Zamenhof and Chargaff (*loc. cit.*) has been obtained by graphical representation of the latter. Thus, two preparations of the "cores" from calf-thymus deoxypentosenucleic acid (which amounted

to 53 and 59·5% of the weight of the original nucleic acid) showed increases in the purine : pyrimidine ratio compared with the original nucleic acid of 12·5 and 10% respectively. Corresponding figures from Zamenhof and Chargaff's results are 9·5 and 6·7%.

Similar increases in the purine : pyrimidine ratio are observed with the cores prepared

TABLE 3.

| Source of non-diffusible "core" and original nucleic acid (DNA) | Mols. of bases per 4 atoms of phosphorus | | | | | Mols. of purine bases/mols. of pyrimidine bases | Purine-N / Pyrimidine-N |
|---|---|---|---|---|---|---|---|
| | A | G | Cy | T | MC | | |
| Calf thymus "core" (53%) ... | 0·94 | 0·80 | 0·65 | 0·89 | 0·07 | 1·08 | 2·23 |
| Calf thymus "core" (59·5%) | 0·98 | 0·75 | 0·72 | 0·85 | 0·07 | 1·06 | 2·13 |
| Calf thymus "core" (20%) ... | 0·98 | 0·74 | 0·63 | 0·91 | 0·05 | 1·08 | 2·23 |
| Calf thymus (core" (22%) ... | 1·05 | 0·75 | 0·65 | 0·99 | 0·05 | 1·07 | 2·19 |
| Calf thymus DNA ..... | 0·99 | 0·77 | 0·76 | 1·00 | 0·07 | 0·96 | 1·98 |
| *M. phlei* "core" (67%) ..... | 0·53 | 0·99 | 1·09 | 0·52 | — | 0·94 | 1·76 |
| *M. phlei* "core" (24%) ..... | 0·41 | 0·89 | 0·86 | 0·51 | — | 0·95 | 1·80 |
| *M. phlei* DNA ..... | 0·57 | 1·00 | 1·10 | 0·49 | — | 0·98 | 1·85 |
| *M. tuberculosis* "core" (54%) | 0·50 | 0·93 | 1·07 | 0·60 | — | 0·86 | 1·62 |
| *M. tuberculosis* "core" (24%) | 0·45 | 0·68 | 0·88 | 0·48 | — | 0·83 | 1·56 |
| *M. tuberculosis* DNA ..... | 0·52 | 0·76 | 0·94 | 0·47 | — | 0·91 | 1·70 |
| Wheat germ "core" (20%) ... | 1·10 | 0·86 | 0·69 | 1·03 | 0·17 | 1·04 | 2·12 |
| Wheat germ DNA ..... | 0·90 | 0·67 | 0·65 | 0·91 | 0·19 | 0·88 | 1·80 |
| Herring testis "core" (50%) | 1·14 | 0·91 | 0·83 | 1·16 | 0·06 | 1·00 | 2·05 |
| Herring testis DNA ..... | 1·00 | 0·71 | 0·82 | 1·05 | 0·10 | 0·87 | 1·74 |
| Mouse sarcoma "core" (50%) | 1·04 | 0·71 | 0·69 | 1·08 | — | 0·99 | 2·06 |
| Mouse sarcoma DNA ..... | 0·95 | 0·70 | 0·73 | 0·82 | — | 1·06 | 2·16 |

A = Adenine, G = guanine, Cy = cytosine, T = thymine, MC = 5-methylcytosine, N = nitrogen. The "cores" are expressed as percentages of the original nucleic acids.

from herring testis, wheat germ, and possibly mouse sarcoma. This increase in the purine : pyrimidine ratio is not, however a characteristic of the larger polynucleotide fragments resulting from the action of deoxyribonuclease on all deoxypentosenucleic acids, since this ratio is slightly reduced in the "cores" obtained from the bacterial deoxypentosenucleic acids.

## EXPERIMENTAL

*Chromatographic Analysis of the Deoxypentosenucleic Acids.*—(a) *Standard materials.* Adenine (supplied by B.D.H. Ltd.) was purified by recystallisation from hot water (Found : N, 52·2. Calc. for $C_5H_5N_5$ : N, 51·8%). Guanine (supplied by B.D.H. Ltd.) was recrystallised according to the procedure of Horbaczewski (*Z. physiol. Chem.*, 1897, **23**, 229) (Found : N, 45·9. Calc. for $C_5H_5ON_5$ : N, 46·5%). Hypoxanthine was prepared by deamination of adenine (Reichard, *J. Biol. Chem.*, 1949, **179**, 773) and recrystallised twice from hot water (Found : N, 41·4. Calc for $C_5H_4ON_4$ : N, 41·2%). Xanthine, prepared from guanine by Reichard's method (*loc. cit.*), exhibited an ultra-violet absorption spectrum identical with that reported in the literature. A sample of cytosine kindly provided by Dr. D. L. Woodhouse was purified by recrystallisation from water (Found : N, 37·7. Calc. for $C_4H_5ON_3$ : N, 37·8%). 5-Methylcytosine was synthesised according to Wheeler and Johnson (*Amer. Chem. J.*, 1904, **31**, 591). After recrystallisation from hot water, the base was obtained as prismatic crystals, m. p. 270° (decomp.) (Found : N, 33·1. Calc. for $C_5H_7ON_3$ : N, 33·6%). *iso*Cytosine was recrystallised twice from hot water and showed m. p. 275—280° (Found : N, 37·9. Calc. for $C_4H_5ON_3$ : N, 37·8%) (Caldwell and Kline, *J. Amer. Chem. Soc.*, 1940, **72**, 2365, give m. p. 276°). Uracil (supplied by B.D.H. Ltd.) was recrystallised from 0·1N-hydrochloric acid (Found : N, 25·3. Calc. for $C_4H_4O_2N_2$ : N, 25·0%). Thymine (supplied by B.D.H. Ltd.) was recrystallised twice from water (Found : N, 22·8. Calc. for $C_5H_6O_2N_2$ : N, 22·3%). Except where otherwise stated, the m. p. of the above bases were as reported in the literature.

(*b*) *Identification and estimation of the amounts of purines and pyrimidines on the chromatograms.* All chromatograms were run on Whatman No. 1 filter paper with a mixture of *n*-butanol, 0·5N-hydrochloric acid, and 2-methoxyethanol (30·5 : 17·7 : 10·1, v/v/v) as solvent. The positions of the bases on the chromatograms were determined by the ultra-violet photographic procedure (Markham and Smith, *Biochem. J.*, 1949, **45**, 295) (for the detection of small amounts of

5-methylcytosine ultra-violet light of wave-length 265 mμ was used). The following $R_F$ values were determined for the standard materials in the above solvent mixture :

| Base : | Adenine | Guanine | Hypoxanthine | Xanthine | Cytosine |
|---|---|---|---|---|---|
| $R_F$ ..... | 0·49 | 0·345 | 0·43 | 0·52 | 0·49 |

| Base : | 5-Methylcytosine | *iso*Cytosine | Thymine | Uracil |
|---|---|---|---|---|
| $R_F$ ..... | 0·54 | 0·54 | 0·77 | 0·685 |

After the spots had been located on the chromatograms by means of the photographic print, the appropriate areas of the paper were cut out and divided into halves. Corresponding areas of paper were cut out from regions on either side of the spot containing the base. The control areas and the purine and pyrimidine spots were cut into small pieces and extracted with 0·1N-hydrochloric acid at 37° for 24 hours. The ultra-violet absorption spectra of the extracts were then determined in the Unicam spectrophotometer. The extracts from the control spots served to correct for any other absorbing material present in the paper and hydrochloric acid. The extinction values were measured at the experimentally determined absorption maxima of the standard bases (in 0·1N-hydrochloric acid) (*i.e.*, adenine at 262·5, guanine at 249, cytosine at 276, 5-methylcytosine at 282, and thymine at 264 mμ). In addition the absorption of the extracts was measured at different wave-lengths (220—290 mμ) in order to effect a comparison between the spectra of the extracts and of the corresponding standard purine and pyrimidine solutions. From the extinction coefficient at the absorption maxima the amount of base in a given extract was calculated from the formula given by Chargaff *et al.* (*J. Biol. Chem.*, 1948, **176**, 703). Since adenine and cytosine had the same $R_F$ value in the solvent mixture used and were thus at the same position on the chromatograms, they were determined from the extinction values at 262·5 mμ (maximum absorption of adenine) and 275 mμ (maximum absorption of cytosine) according to formulæ derived from those given by Chargaff *et al.* (*ibid.*, 1950, **186**, 37; cf. Loring and Ploeser, *ibid.*, 1949, **178**, 439).

(c) *Procedure for the complete chromatographic analysis of the deoxypentosenucleic acids.* The deoxypentosenucleic acid (5 mg.) was heated in a sealed tube (10 × 0·6 cm. internal diam; wall thickness, 2·5 mm.) with formic acid ("AnalaR;" 0·6 c.c.) for 50 minutes at 175—180°. Aliquots (0·05 c.c.) of the dark brown hydrolysate were placed on filter paper strips (10 × 52 cm.). Five chromatograms were developed for each hydrolysate. If no 5-methylcytosine was present a separation sufficient for the estimation of adenine, guanine, cytosine, and thymine was achieved after 15 hours at 18—20° (total length of flow, 40 cm.). However, if preliminary experiments revealed the presence of 5-methylcytosine adequate resolution was only obtained after 24 hours (total length of flow, 60—65 cm.). The chromatograms were dried in a stream of warm air, the positions of the bases were located by ultra-violet photography, and the quantitative estimations then performed as described above.

When small amounts of 5-methylcytosine were present in the hydrolysates the appropriate segments from 3—4 chromatograms were combined and extracted with 0·1N-hydrochloric acid (5 c.c.). The amount of the base present in the extract was calculated from the ultra-violet absorption measurements at 282 mμ.

The following results obtained from the analysis of calf-thymus deoxypentosenucleic (prep. $T_1$) are recorded as illustrative of the accuracy obtained with the chromatographic method of analysis : Three main spots were visible on the chromatogram corresponding to guanine, adenine + cytosine, and thymine. Estimation of the bases in these spots gave the following results (in μg.) :

| | Chromatogram number | | | | |
|---|---|---|---|---|---|
| Base : | 1 | 2 | 3 | 4 | 5 |
| Thymine ..... | 43·2 | 45·8 | 40·5 | 39·0 | 38·0 |
| Guanine ..... | 44·0 | 39·5 | 39·5 | 37·5 | 38·7 |
| Adenine ..... | 43·8 | 43·0 | 44·2 | 43·5 | 45·0 |
| Cytosine ..... | 28·5 | 27·2 | 27·2 | 29·2 | 28·7 |

5-Methylcytosine (estimated by combining four chromatograms) = 3·25 μg.

*Enzymic Degradation of the Deoxypentosenucleic Acids.*—A solution (0·14%; 1 vol.) of the deoxypentosenucleic acid (of known phosphorus content), 0·1M-veronal buffer (0·5 vol.; pH 7·1) containing 0·01M-$Mg^{\cdot\cdot}$, and a solution (0·01%; 0·5 vol.) of deoxyribonuclease (isolated from pancreas according to McCarty's method, *J. Gen. Physiol.*, 1946, **29**, 123) were incubated together at 37° for 15 hours. The enzyme digest was then dialysed at room temperature in a Cellophane bag against distilled water (1 l. per 10 c.c. of digest) containing 0·0002% of merthiol-

ate. The external aqueous phase was changed after 7, 24, 48, 72, and 144 hours. The combined dialysates were evaporated and analysed for total phosphorus. The non-dialysable " cores " were obtained by evaporation of the solution remaining in the dialysis sac in the frozen state. In Table 3 these " cores " are characterised as percentages of the original nucleic acids (based on the phosphorus contents of the dialysates and the original nucleic acids).

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## 618. *The Polarographic Reduction of Crystal-violet, Brilliant-green, Malachite-green, and Auramine.*

By R. C. KAYE and H. I. STONEHILL.

All four dyes are reducible in aqueous solution at the dropping mercury electrode, giving polarograms distorted by dye adsorption on the mercury. When adsorption effects are eliminated by addition of ethanol, normal polarograms result, exhibiting two well-separated one-electron steps which indicate the formation at the first step of a semiquinone free radical of apparently very great stability. For all but malachite-green, which was not studied in sufficient detail to establish the point, the half-wave potential of the first reduction step is independent of pH in the biologically important region around pH 7, so that, as with the more strongly antibacterial aminoacridines (*J.*, 1951, 2638), semiquinone formation involves the uptake of one electron and no proton. Equations are derived which represent satisfactorily the observed variation of half-wave potential with pH.

PREVIOUS work on the polarographic reduction of acridine (*J.*, 1951, 27) and several aminoacridines (*J.*, 1951, 2638) has shown that, while all these compounds give two widely separated one-electron reduction steps, indicating the formation of a semiquinone free radical of exceptionally great apparent stability, the more strongly antibacterial of them alone have a horizontal portion of the $E_1$–pH curve ($E_1$ = half-wave or effective standard redox potential of first step) extending over the biologically important pH region around 7. This horizontal section indicates that the formation of semiquinone involves the uptake of a single electron but no proton, a matter of significance in connection with a theory of the mechanism of these antibacterials (Kaye, *J. Pharm. Pharmacol.*, 1950, **2**, 902).

In order to find whether the antibacterial triphenylmethane dyes crystal-violet, brilliant-green, and malachite-green, and the bacteriostatic diphenylmethane dye auramine behave similarly to the active aminoacridines, we have investigated their polarographic reduction. We are not aware of any previous comprehensive polarographic studies of these dyes, although Linnell and Stenlake (*J. Pharm. Pharmacol.*, 1949, **1**, 314) confirmed polarographically the identity of a derivative of brilliant-green.

### EXPERIMENTAL

Crystal-violet and brilliant-green of medicinal purity, and commercial malachite-green and auramine, were purified by recrystallisation from water; for the first two, prolonged standing of the solutions was necessary. All other materials, apparatus, and technique were as previously described (*J.*, 1951, 27). The temperature of all experiments was 25°, all polarograms were corrected for residual current, and electrode potentials are relative to the saturated calomel electrode standard.

### RESULTS AND DISCUSSION

*Crystal-violet.*—In aqueous solution this dye is electro-reducible at the dropping mercury electrode, giving two-step reduction waves (Fig. 1) which are distorted, especially at the second step. The distortions were associated with anomalous galvanometer

oscillations, as with acridines, and are thus ascribed to adsorption of electroactive matter on the surface of the dropping mercury electrode. This is confirmed by current–time oscillograms taken during a single drop life (cf. *J.*, 1951, 27, 2638). Abnormal oscillograms were obtained only at potentials corresponding to the fore-wave of the second step, and their shape suggests that current growth is prevented by fully reduced material blocking the mercury surface. The inclusion of 50% by volume of ethanol in the base (supporting electrolyte) solution eliminated the adsorption effects in the polarogram (Fig. 1), the waves becoming normal apart from a tendency at certain pH values to maximum formation. This was suppressed by adding 0·01—0·02% of methyl cellulose, without affecting wave height. The presence of 50% of ethanol did not, however, completely remove the oscillogram anomalies, showing that, as with the aminoacridines, the oscillogram provides a more sensitive test for adsorption on the mercury drop than does the polarogram.

The dye was examined polarographically in 50% aqueous ethanol, at a concentration of $4 \times 10^{-4}$M, over the pH range 0—14. Between pH 1 and pH 14, the two-step reduction waves were normal and of constant height (for fixed capillary characteristics), the diffusion current constant being very nearly equal to that of the acridines in the same solvent, but only two-thirds of the value for the dye in aqueous solutions. Application of the Ilkovič

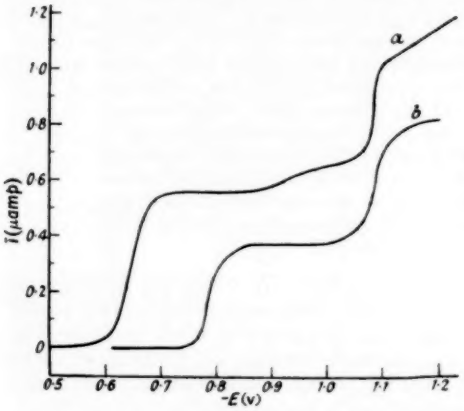



FIG. 1. *Polarograms for crystal-violet,* $4 \times 10^{-4}$M. *a,* pH 7·38, *in water; b,* pH 8·29, *in* 50% *ethanol.*

equation $\bar{i}_d = 605ncD^{\frac{1}{2}}m^{\frac{2}{3}}t^{\frac{1}{6}}$ (*Coll. Czech. Chem. Comm.*, 1934, **6**, 498) to the results at pH 8·29 in 50% ethanol, with the values $D^{\frac{1}{2}}$ ($D$ = diffusion coefficient of reducible molecule) = $1{\cdot}52 \times 10^{-3}$ cm. sec.$^{-\frac{1}{2}}$, $m$ (mercury flow rate) = 0·916 mg. sec.$^{-1}$, $c$ (concentration) = 0·4 millimol./l., $t$ (drop time) = 4·1 sec. (first step) and 4·0 sec. (second step), $\bar{i}_d$ (mean diffusion current) = $0{\cdot}44 \times 10^{-3}$amp (first step) and $0{\cdot}46 \times 10^{-3}$amp (second step), gave the results $n$ (number of electrons involved per molecule) = 1·002 (first step) and 1·06 (second step). The foregoing value of $D^{\frac{1}{2}}$ was obtained from the value $D = 5{\cdot}2 \times 10^{-6}$ cm.$^2$ sec.$^{-1}$ for the phenolphthalein molecule (of similar size to that of crystal-violet) in water at 25° (Kolthoff and Lehmicke, *J. Amer. Chem. Soc.*, 1948, **70**, 1879) by multiplying its square root by $\frac{2}{3}$ to allow for the effect of the alcohol on wave height, it being assumed (cf. Shreve and Markham, *ibid.*, 1949, **71**, 2993) that the presence of alcohol does not affect the value of $m^{\frac{2}{3}}t^{\frac{1}{6}}$. Thus the two steps are single-electron reductions, and the product of the first stage is a semiquinone free radical, the stability of which is apparently very great, as indicated by the wide separation of the half-wave potentials $E_{\frac{1}{2}}$ and $E_{\frac{1}{2}}$, which may be taken as equal to the standard redox potentials $E_1$ and $E_2$ of the two steps. An alternative indication is the large value ($10^{3{\cdot}75}$) at pH 7 of the semiquinone formation constant

$$K = [\text{semiquinone}]^2/[\text{unreduced dye}][\text{fully reduced dye}] \quad . \quad . \quad . \quad . \quad . \quad (1)$$

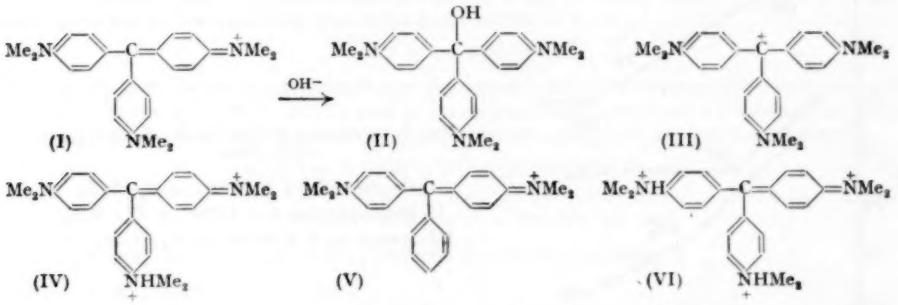
calculated from the equation (Michaelis, *Ann. N.Y. Acad. Sci.*, 1940, **40**, 39)

$$K^{\frac{1}{2}} = 10^{E_1/0{\cdot}059} - 3 \times 10^{-E_1/0{\cdot}059} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where $E_i$ is the index potential defined in equation (17). Although these equations apply strictly only to reversible systems, the values of $K$ obtained from them still retain a qualitative significance even for irreversible systems, as, *e.g.*, the aminoacridines.

At pH $\geqslant$11, the wave-heights of both steps diminished at a measurable rate when the solution was kept, the dye colour meanwhile fading. The rate of the change to a colourless, non-reducible form of the dye increased with rise of pH. Thus at pH 13 the conversion was so rapid that it was impossible to prepare a solution for polarography before it had changed completely to irreducible material. A similar but faster time-decrease of wave-height occurred at pH $\leqslant$1, rapidly yielding an equilibrium in which the extent of conversion to non-reducible matter, measured by the decrease in wave-height, increased as pH decreased and was almost 100% at pH 0. This conversion was also accompanied by change of colour, to green at pH 1—0, greenish-yellow at pH 0, and yellow at greater acidities.

The change at pH $\geqslant$11 is due to conversion of the violet form into the colourless carbinol (II) or, in the presence of ethanol, into the corresponding ether (Goldacre and Phillips, *J.*, 1949, 1724; Fischer, *Ber.*, 1900, **33**, 3356). Neither carbinol nor ether has a carbonium-




ion resonance contribution. This suggests that the emergence of electroreducibility and violet colour below pH 11 may be ascribed to resonance between the carbonium-ion structure (III) and either three equivalent structures (I) or, if the three rings are not all coplanar (Lewis, Magel, and Lipkin, *J. Amer. Chem. Soc.*, 1942, **64**, 1774), two such structures. The carbonium-ion structure would seem to confer electroreducibility because of the electron-deficient central carbon atom. At about pH 1, the colour change to green is probably due to acquisition of a proton by one nitrogen atom, as in (IV), which resembles the structure of malachite-green (V) in having only two nitrogen atoms and their associated rings conjugated; resonance with a carbonium-ion structure is still possible, however, and electroreducibility is still observed. At about pH 0, a second proton is added (cf. Adams and Rosenstein, *ibid.*, 1914, **36**, 1452), giving structure (VI), which presumably accounts for the yellow colour. Since no further addition beyond the second proton occurs even in 100% sulphuric acid (Newman and Deno, *ibid.*, 1951, **73**, 3644; Branch and Tolbert, *ibid.*, 1949, **71**, 781), the quinonoid ring in (VI) is very resistant to conversion into a benzenoid ring by accession of a proton to the nitrogen atom with consequent change to carbonium-ion structure. Thus, (VI) is apparently unable to resonate with a carbonium-ion structure (cf. Newman and Deno, *loc. cit.*), perhaps because of repulsion of like charges, and therefore is not electroreducible.

Over the pH range 1—11, where crystal-violet exhibits the violet colour and normal electroreducibility, the variation of $E_1$ and $E_2$ with pH is as shown in Fig. 2. The $E_1$–pH curve has a bend at pH 4·2, the direction of which indicates (Clark, *Chem. Reviews*, 1925, **2**, 127) that the semiquinone has an acid dissociation constant $K^s$ such that $pK^s = 4{\cdot}2$. No bend appears in the $E_1$–pH curve corresponding to the acid exponent $pK = 9{\cdot}36$ found by Goldacre and Phillips (*loc. cit.*), for the carbinol form of crystal-violet in water at 25°, probably because in 50% ethanol the change to carbinol is too slow at pH <11 and would in

any case yield the ether rather than the carbinol itself. Since the $E_1$–pH slope is −103 mv below, and zero above, pH 4·2, the first stage of the reduction would appear to be

at pH >4·2 : $\{Me_2N-C_6H_4-\}_3\overset{+}{C} + e \longrightarrow \{Me_2N-C_6H_4-\}_3\dot{C}$ (VII) . . (3)

at pH <4·2 : $\{Me_2N-C_6H_4-\}_3\overset{+}{C} + e + H^+ \longrightarrow \{Me_2N-C_6H_4-\}_2\dot{C}-C_6H_4-\overset{+}{N}HMe_2$ (VIII) (4)

The last equation would indicate an $E_1$–pH slope of −59 mv if the process concerned were reversible. However, a plot of $\log\{(\bar{i}_d - \bar{i})/\bar{i}\}$ against $E$ ($\bar{i}$ = average polarographic current at dropping mercury electrode potential $E$; $\bar{i}_d$ = average diffusion current) gave curves of varying slope for both reduction steps, indicating thermodynamic irreversibility. If it were assumed that the observed −103 mv slope corresponded to uptake of two protons,

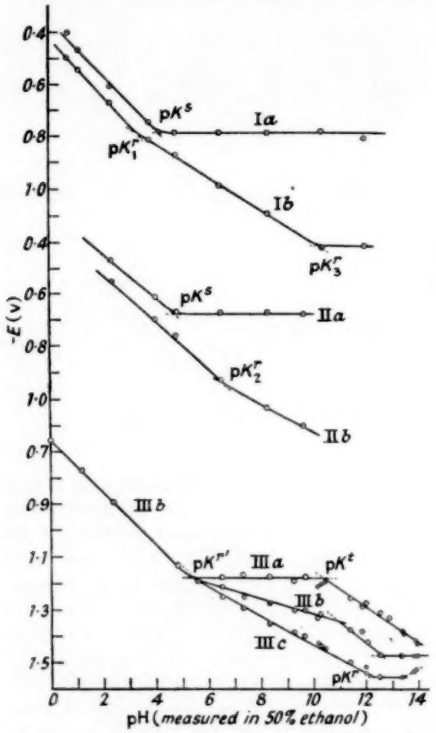



FIG. 2. *Variation with* pH *of* $E_1$, $E_2$, *and* $E_m$ *in* 50% *ethanol.*

I, *Crystal-violet*, 4 × $10^{-4}$M : *a*, $E_1$; *b*, $E_2$.
II, *Brilliant-green*, 4 × $10^{-4}$M : *a*, $E_1$; *b*, $E_2$.
III, *Auramine*, 4 × $10^{-4}$M : *a*, $E_1$; *b*, $E_m$; *c*, $E_2$.

it would then be necessary to suppose that the semiquinone had two p$K^s$ values close to each other and in the neighbourhood of 4·2.

If reaction (3) is treated as if reversible, with standard redox potential $E_1^0$, the corresponding electrode potential in v at 25° is

$$E = E_1^0 + 0{\cdot}059 \log [Ox]/[S] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

where Ox denotes the sole ionic form (III ⟷ I) of unreduced dye existing over pH range 1—11, and S denotes the semiquinone radical (VII). If (VIII) is denoted by $S^+$, we have the ionisation equilibrium

$$S^+ \rightleftharpoons S + H^+, \; K^s = [S][H^+]/[S^+] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

whence the total concentration of semiquinone in both forms is

$$[Sem] = [S] + [S^+] = [S]\{1 + [H^+]/K^s\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

By substitution for [S] in terms of [Sem] from equation (7), and reference to the half-wave potential, where $E = E_1$ and [Ox] = [Sem], equation (5) becomes

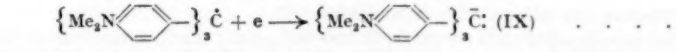
$$E_1 = E_1^0 - 0{\cdot}059 \log K^s + 0{\cdot}059 \log\{K^s + [H^+]\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

According to this equation, the $E_1$–pH curve should have zero slope for pH $>$ p$K^s$, and a slope of $-59$ mv for pH $<$ p$K^s$. Apart from the numerical discrepancy in the latter slope, ascribed to irreversibility, the experimental curve is in conformity with this. A similar treatment, based on the assumption that $S^+$ (acid constant $K_2^s$) may add a further proton to give $S^{++}$ (with acid constant $K_1^s$), leads to

$$E_1 = E_1^0 - 0{\cdot}059 \log K_1^s K_2^s + 0{\cdot}059 \log \{K_1^s K_2^s + K_1^s[H]^+ + [H^+]^2\} \quad . \quad . \quad . \quad (9)$$

If $K_1^s \simeq K_2^s$, a possibility suggested above, equation (9) indicates an $E_1$–pH slope of zero for pH $>$ p$K_{1,2}^s$, and $-118$ mv for pH $<$ p$K_{1,2}^s$. The experimental slope of $-103$ mv is certainly nearer to the requirements of equation (9) than those of equation (8), but there is good reason (see below) to prefer equation (8).

The zero slope of the $E_2$–pH curve, at pH $>10{\cdot}3$ indicates that the second reduction step does not involve uptake of a proton. It is therefore represented by



$$\{Me_2N\text{–}C_6H_4\text{–}\}_3\dot{C} + e \longrightarrow \{Me_2N\text{–}C_6H_4\text{–}\}_3\bar{C}: \text{ (IX)} \quad . \quad . \quad . \quad . \quad (10)$$

leading to the formation of an anion $R^- =$ (IX) similar to that postulated by us and by other workers in other comparable reductions (*J.*, 1951, 27, 2638). The electrode potential corresponding to reaction (10), treated as if reversible, is

$$E = E_2^0 + 0{\cdot}059 \log [S]/[R^-] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

We assume that the semiquinone exists in the forms S and $S^+$ as before, while fully reduced dye exists in the forms $R^{++}$, $R^+$, R, and $R^-$, corresponding to successive removal of protons, according to the following processes, the last of which is almost certainly not reversible :

$$R^{++} = R^+ + H^+; \; K_1^r = [R^+][H^+]/[R^{++}] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

$$R^+ = R + H^+; \; K_2^r = [R][H^+]/[R^+] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

$$R = R^- + H^+; \; K_3^r = [R^-][H^+]/[R] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

Then the total concentration of fully reduced dye in all four forms is

$$[\text{Red}] = [R^-] + [R] + [R^+] + [R^{++}]$$
$$= [R^-]\{K_1^r K_2^r K_3^r + K_1^r K_2^r [H^+] + K_1^r [H^+]^2 + [H^+]^3\}/\{K_1^r K_2^r K_3^r\} \quad . \quad . \quad . \quad (15)$$

By substitution for $[R^-]$ in terms of [Red] from equation (15), and for [S] in terms of [Sem] from equation (7), and reference to the half-wave potential where $E = E_2$ and [Sem] = [Red], equation (11) becomes

$$E_2 = E_2^0 + 0{\cdot}059 \log K^s/K_1^r K_2^r K_3^r + 0{\cdot}059 \log \{K_1^r K_2^r K_3^r + K_1^r K_2^r [H^+]$$
$$+ K_1^r [H^+]^2 + [H^+]^3\}/\{K^s + [H^+]\} \quad . \quad . \quad . \quad (16)$$

With $K_1^r > K_2^r > K_3^r$, equation (16) would indicate, in the absence of the denominator $K^s + [H^+]$ of the last term, an $E_2$–pH graph with linear sections of slopes $-177$ mv for pH $<$ p$K_1^r$, $-118$ mv for pH between p$K_1^r$ and p$K_2^r$, $-59$ mv for pH between p$K_2^r$ and p$K_3^r$, and zero for pH $>$ p$K_3^r$. Thus, with increasing pH, at each p$K^r$ value the slope should decrease numerically by 59 mv. Similarly the denominator $K^s + [H^+]$ should cause a numerical increase of 59 mv in the slope at pH $=$ p$K^s$. If now it were assumed that p$K^s \simeq$ p$K_2^r$, the opposed effects on the slope of the bends at these two p$K$ values would annul each other, leaving a deceptively simple graph with slope $-118$ mv for pH $<$ p$K_1^r$, $-59$ mv for pH between p$K_1^r$ and p$K_3^r$, and zero for pH $>$ p$K_3^r$. A plot of equation (16) with the experimental values p$K_1^r = 3{\cdot}4$, p$K_2^r =$ p$K^s = 4{\cdot}2$, p$K_3^r = 10{\cdot}3$, and $E_2^\circ + 0{\cdot}059 \log K^s/K_1^r K_2^r K_3^r$ (extrapolated value of $E_2$ at pH $= 0$) $= -0{\cdot}400$ v reproduces the experimental graph very well except for the discrepancy in slopes ($-62$ and $-107$ instead of $-59$ and $-118$ mv) due to irreversibility. If it were assumed, as suggested above in connexion with the $E_1$–pH curve, that the semiquinone could exist in the form $S^{++}$ as well as S and $S^+$, a similar treatment would lead to the equation

$$E_2 = E_2^0 + 0{\cdot}059 \log K_1^s K_2^s/K_1^r K_2^r K_3^r$$
$$+ 0{\cdot}059 \log \{K_1^r K_2^r K_3^r + K_1^r K_2^r [H^+] + K_1^r [H^+]^2 + [H^+]^3\}/\{K_1^s K_2^s + K_2^s [H^+] + [H^+]^2\}$$

According to this equation, the $E_2$–pH slope could not exceed numerically $-59$ mv between pH 1 and pH 11, if $K_1^r \gg K_1^s \simeq K_2^s \simeq K_2^r \gg K_3^r$.

The facts that the $E_1$–pH graph is horizontal in the region of pH 7, and that the semiquinone is apparently highly stable in this region ($E_i \simeq E_1 - E_2 = 0{\cdot}111$ v, $K = 10^{3{\cdot}75}$ at pH 7), show that this dye has redox properties similar to those of the more actively antibacterial aminoacridines.

*Brilliant-green.*—This dye also gave two-step polarograms in water, distorted by adsorption, which was eliminated by inclusion of 50% of ethanol in the solvent. $4 \times 10^{-4}$M-solutions of the dye in 50% ethanol were examined polarographically over the pH range 0—14. At pH >9, the dye underwent progressive fading and conversion into irreducible form as alkalinity increased, the solutions becoming turbid; the conversion rate increased with pH. These effects are ascribed to carbinol formation, as with crystal-violet. Conversion into irreducible form also occurred at pH below about 2, and was almost complete at pH 1, but little change of colour was observed until pH 0 was reached, the solution then becoming pale green-yellow. These changes may be explained similarly to the corresponding effects with crystal-violet.

In the intermediate pH range 2—9, the diffusion current constants of the two steps were independent of pH and slightly smaller than for crystal-violet. By applying the Ilkovič equation with the same values of $D$, $m$, $t$, and $c$ as were used for crystal-violet, and with $i_d = 0{\cdot}35$ μamp (first step), 0·38 μamp (second step), the values $n = 0{\cdot}79$ (first step) and 0·86 (second step) are obtained, confirming that each step involves one electron. The product of the first reduction step is thus a semiquinone radical, the apparent stability of which, at pH >5, is considerable, to judge from the wide separation of the two steps.

The $E_1$–pH curve (Fig. 2) is of the same form as that for crystal-violet, and the same interpretation (equation 8) is applicable, with $pK^s = 4{\cdot}8$, a somewhat greater value than for crystal-violet, in conformity with the superior electron-repellency of ethyl compared with methyl, which renders the nitrogen atom more strongly basic in $(p\text{-Me}_2\text{N}\cdot\text{C}_6\text{H}_4)_2\dot{\text{C}}\text{Ph}$ than in (VII). The slope of the graph is zero in the region of pH 7, but at pH $< pK^s$ it is −87 mv instead of −59 mv required by equation (8), presumably because of the irreversibility of electroreduction. No bend appears in the $E_1$–pH graph corresponding to the p$K$ value of 7·90 reported for pure aqueous solutions of brilliant-green at 25° by Goldacre and Phillips (*loc. cit.*), probably for the same reasons as in the case of crystal-violet.

The $E_2$–pH graph (Fig. 2) is similar to that for crystal-violet, but lacks the horizontal section at extreme alkalinities. This section, starting from a bend at pH $= pK_3^r \simeq$ 10—11, would probably have been obtained, had not carbinol formation prevented polarographic investigation at pH >9·5. In the pH range 6·5—10, the graph has a −52 mv slope, corresponding to the equation

$$S + e + H^+ \longrightarrow R$$

for the electrode reaction. By applying the method used in deriving equation (16) for crystal-violet, but omitting $R^-$ and $K_3^r$, an equation is obtained which is similar to equation (16) except for the omission of the term $K_1^r K_2^r K_3^r$ wherever it occurs. With the assumptions that $pK_2^r = 6{\cdot}5$ (corresponding to the $E_2$–pH bend at pH 6·5), and that $pK^s \simeq pK_1^r$ (so that the opposing bends associated with these acid constants mutually cancel), the equation indicates that the $E_2$–pH graph should have slopes −118 mv for pH $< pK_2^r$, and −59 mv for $pK_2^r >$ pH $> pK_1^r$. The experimental slopes of −90 and −52 mv are in approximate agreement with theory, the discrepancy again being ascribed to irreversibility of the reduction. That $pK_1^r$, $pK_2^r$, and perhaps also $pK_3^r$ are all greater for this dye than for crystal-violet is to be expected because of the greater electron repellency of ethyl than that of methyl.

Brilliant-green, like crystal-violet, has a horizontal $E_1$–pH plot and a high semiquinone apparent stability at pH 7 ($E_i \simeq E_1 - E_2 = 0{\cdot}141$ v, $K = 10^{4{\cdot}75}$), thus resembling the more actively antibacterial aminoacridines.

*Malachite-green.*—This was examined at two pH values only. Its behaviour was practically indistinguishable from that of brilliant-green, and therefore no further work was carried out on it.

*Auramine.*—This dye gave grossly distorted polarograms in aqueous solution, indicating strong adsorption effects, confirmed by erratic galvanometer oscillations during a drop life,

as with acridine. The inclusion of 50% of ethanol in the supporting electrolyte again removed adsorption irregularities, but the polarograms showed a tendency to exhibit maxima, especially at high concentrations. The addition of 0·01% of methyl cellulose suppressed all maxima, including one at about −1·45 v which appeared as a hump in the pH range 11—13·5 approx. and was progressively exalted on incorporation of increasing amounts of potassium chloride in the solution (Fig. 3). Neither methyl cellulose nor potassium chloride affected the wave-height appreciably. The maxima are probably caused by adsorption of auramine or its reduction product on the mercury drops; this is enhanced by the salting-out effect of potassium chloride, or prevented by the preferential adsorption of methyl cellulose. An alternative view is that the maxima are due to non-uniform polarisation of the mercury-drop surface, with consequent tangential flow of mercury at the surface instead of the radial flow assumed in the derivation of the Ilkovič equation. This view is, however, unlikely since it would lead to the expectation that addition of potassium chloride, by increasing the conductance of the solution, should render the mercury-drop surface more uniformly polarised and thus tend to suppress the maxima.

If attention is restricted to solutions in 50% ethanol, at pH >5·3 the polarograms exhibited two steps, with maximum separation at pH 10, and with diffusion current constants independent of pH. The wave-heights of the first and second steps for $4 \times 10^{-4}$M-auramine at pH 12 were 0·439 and 0·449 μamp respectively, which are not very

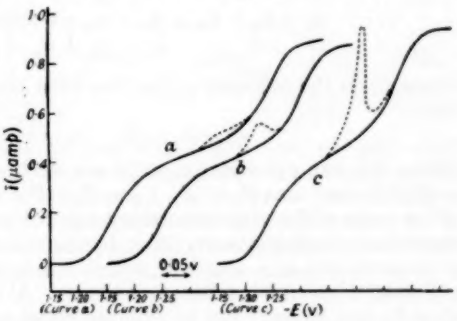
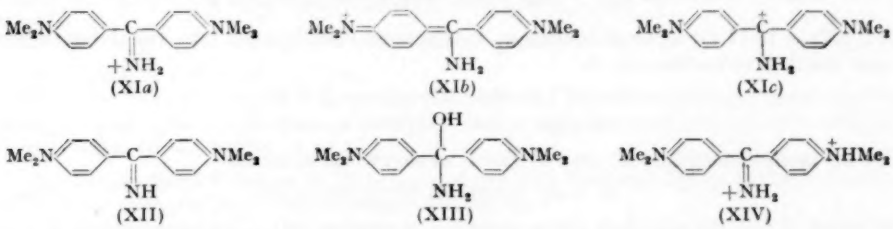



FIG. 3. *Polarograms for auramine,* $4 \times 10^{-4}$M *in 50% ethanol,* pH 12·0.
*a, No potassium chloride.*
*b,* 0·1N-*Potassium chloride.*
*c,* N-*Potassium chloride.*

different from the values given for crystal-violet in the same solvent with the same values of $c$, $m$, and $t$. If $D$ is assumed to have roughly the same value for both dyes, the Ilkovič equation indicates that $n = 1$ for each step in the auramine reduction. At pH below about 5·3, the two steps merged into a single two-electron step. Between pH 5 and pH 7 the auramine reduction wave was followed immediately by a catalytic hydrogen wave, which rendered the polarograms ill-defined and unreliable. At pH above 7, this catalytic wave shifted to potentials sufficiently different from those of the second auramine step (about 0·7 v more negative) to preclude any interference with the latter. At the same time the catalytic wave height decreased with rise of pH, reaching zero at about pH 12·5.




Auramine (XI*a*), unlike the other dyes examined, is electroreducible over the entire pH range 0—14. Its yellow colour becomes greener near pH 0, and at pH above about 12 it is colourless, as also at pH well below 0. Goldacre and Phillips (*loc. cit.*) state that, upon

treatment with alkali, auramine loses a proton to give the imine (XII) with a p$K$ of 10·71 in water at 25°; the imine takes up water only very slowly to give the colourless carbinol (XIII), which by analogy with crystal-violet carbinol is probably irreducible. We assume that the colour of auramine is due to resonance with structures such as (XI*b*), and its electroreducibility to resonance with the carbonium-ion structure (XI*c*), as with crystal-violet. Addition of a proton gives (XIV), in which reducibility persists and colour is only modified to greener shades, because both types of resonance required are still possible, although one is decreased in extent by the nitrogen atom's acquiring a proton. Further addition of protons would inhibit completely the resonance associated with the colour, and thus does not occur unless the pH is well below 0. In the imine, both types of resonance are inhibited because they would necessitate a separation of opposite charges. Thus, as in Michler's ketone ($p$-$Me_2N{\cdot}C_6H_4)_2CO$, colour disappears, but electroreducibility remains because there is still a centre of positive charge at the central carbon atom owing to the considerable polarisation of the double bond $\overset{\delta+}{C}{=}\overset{\delta-}{N}H$.

In connexion with the $E_1$–pH and $E_2$–pH graphs for auramine (Fig. 2), it is necessary to distinguish between the standard redox potentials $E_1$, $E_2$ (at a given pH) for two successive one-electron reduction steps, and the dropping mercury electrode potentials $E_{\frac{1}{4}}$, $E_{\frac{3}{4}}$ at $\frac{1}{4}$ and $\frac{3}{4}$ of the total reduction wave height or extent of reduction. For a reversible system buffered to constant pH, it is readily shown that, in v at 25°,

$$E_1 = E_{\frac{1}{4}} - E_m = E_m - E_{\frac{3}{4}} = 0{\cdot}059 \log K^t + 0{\cdot}059 \log (\tfrac{1}{2} + \sqrt{\tfrac{1}{4} + 3/K}) \quad . \quad (17)$$

$$E_1 - E_m = E_m - E_2 = 0{\cdot}059 \log K^t \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (18)$$

where $E_m$ is the potential at half the total wave height or extent of reduction. It follows that

$$E_{\frac{1}{4}} - E_1 = E_2 - E_{\frac{3}{4}} = 0{\cdot}059 \log (\tfrac{1}{2} + \sqrt{\tfrac{1}{4} + 3/K}) = \delta \quad . \quad . \quad . \quad . \quad (19)$$

When the two one-electron steps are well separated, so that $E_1$ and $K$ are large, $\delta$ is negligible, and thus $E_{\frac{1}{4}} = E_1$, $E_{\frac{3}{4}} = E_2$. For $\delta$ = 1, 2, and 5 mv (the last two values being of the order of the experimental error in potential measurements), $E_i$ = 56, 47, and 37 mv, respectively (equations 17, 19). In the measurements with the triphenylmethane dyes $E_i$ is never less than about 37 mv, so $\delta$ is negligible. For auramine the same is true for pH down to about 6·5, where $E_i$ is 40 mv. At lower pH, however, $E_i$ falls below 37 mv, and thus $E_1$ and $E_2$ must not be assumed equal to $E_{\frac{1}{4}}$ and $E_{\frac{3}{4}}$, but may be calculated from $E_i$ and $E_m$ by means of equations 2 and 18. At pH 5·5 approx., $E_i$ for auramine is 21 mv, corresponding to the point where $E_1 = E_2 = E_m$ and the $E_1$–pH and $E_2$–pH graphs cross. Below about pH 5·3, $E_i$ is constant at 14 mv, corresponding to $K = 0$ or absence of semiquinone formation; this contrasts with the behaviour of the triphenylmethane dyes, which exhibited semiquinone formation over the whole pH range examined, except where they became irreducible at extreme acidity or alkalinity.

The $E_1$–pH graph (Fig. 2) consists of two linear portions with slopes 0 (pH 5·5—10·4) and −73 mv (pH >10·4). If it is assumed that auramine exists in the two forms $T^+$ (= XI) and T (= XII) related by the equilibrium

$$T^+ \rightleftharpoons T + H^+; \; K^t = [T][H^+]/[T^+]; \; pK^t = 10{\cdot}4 \quad . \quad . \quad . \quad . \quad . \quad (20)$$

(cf. p$K^t$ = 10·71 for aqueous solutions; Goldacre and Phillips, *loc. cit.*), the slopes suggest that the first reduction step is

$$\text{For pH 5·5—10·4: } T^+ + e \longrightarrow S(= XV) \quad . \quad . \quad . \quad . \quad . \quad . \quad (21)$$

$$\text{For pH >10·4: } T + H^+ + e \longrightarrow S \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

By the method described for crystal-violet, we derive the equation

$$E_1 = E_1^0 + 0{\cdot}059 \log \{[H^+]/([H^+] + K^t)\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$

in which $E_1^0$ is the standard redox potential of process (21). The experimental $E_1$–pH graph accords with equation (23), apart from the discrepancy between the observed and theoretical (−59 mv) slope for pH >10·4, probably due mainly to irreversibility.

The $E_2$–pH graph (Fig. 2) consists of two linear sections with slopes −53 mv (pH 5·5—

12·2) and 0 (pH 12·2—13·4), followed by an upward bend at pH 13·4. This bend will be ignored, as it is probably due to the change of supporting electrolyte from a weak-acid buffer to sodium hydroxide solution, with consequent increase in ionic strength. This increase is known to make $E_2$ and also $E_1$ more positive; thus, at pH 12·0, the inclusion of 0·1N- and 1·0N-potassium chloride in the supporting electrolyte raised $E_2$ from −1·544 to −1·510 and −1·480 v, and $E_1$ from −1·272 to −1·263 and −1·252 v, respectively. The slopes suggest that the second reduction step is

$$\text{For pH 5·5—12·2: } S + e + H^+ \longrightarrow R\ (= \text{XVI}) \quad . \quad . \quad . \quad . \quad . \quad . \quad (24)$$

$$\text{For pH } > 12\cdot2\text{: } S + e \longrightarrow R^-\ (= \text{XVII}) \quad . \quad . \quad . \quad . \quad . \quad . \quad (25)$$

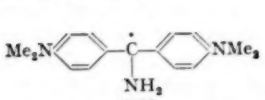
The anionic product $R^-$, which probably abstracts a proton from a water molecule immediately after formation, thus changing to the form R, is analogous to the anionic reduction product postulated for crystal-violet. Assuming that R and $R^-$ are related by the equilibrium

$$R \rightleftharpoons R^- + H^+;\ K^r = [R^-]\,.\,[H^+]/[R];\ pK^r = 12\cdot2 \quad . \quad . \quad . \quad . \quad (26)$$

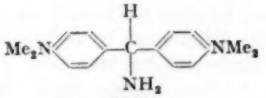
we derive by a familiar method the equation

$$E_2 = E_2^0 + 0\cdot059 \log\{([H^+] + K^r)/K^r\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (27)$$

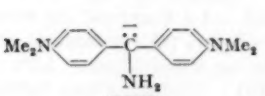
in which $E_2^0$ is the standard redox potential of process (25). Apart from a small discrepancy in slope for pH 5·5—12·2, the experimental $E_2$–pH graph is in conformity with equation (27).



(XV)



(XVI)



(XVII)

The $E_m$–pH graph (Fig. 2) comprises linear sections with slopes −98 mv (pH <5·3) and −29 mv (pH 5·3—10 approx.). At pH >*ca.* 10, the second reduction step was considerably steeper than the first, so that $E_m$, which was always taken as the potential at the point of inflexion between the two steps in order to allow for small differences in the wave-heights, was far from midway between $E_1$ and $E_2$; little theoretical significance could then be attached to $E_m$. For pH 5·3—10 approx., the slope is in accordance with the overall two-electron reduction process

$$T^+ + 2e + H^+ \longrightarrow R$$

which is the sum of processes (21) and (24). The bend in the $E_m$–pH graph at pH 5·3 suggests that for pH <5·3 the reduction product exists mainly in the form $R^+$ resulting from addition of a proton to R, with acid exponent $pK^{r'} = 5\cdot3$ for the associated ionisation. For pH <5·3, the one-stage two-electron reduction process would then be

$$T^+ + 2e + 2H^+ \longrightarrow R^+.$$

This, however, corresponds to a slope of −59 mv. A closer approximation to the observed slope of −98 mv would be obtained by supposing that in this pH range $R^+$ has added a proton at one of the nitrogen atoms to give $R^{++}$, with acid exponent $pK^{r''}$ for the associated ionisation equilibrium only slightly smaller than $pK^{r'}$. The reduction process would then be

$$T^+ + 2e + 3H^+ \longrightarrow R^{++},$$

corresponding to a slope of −87 mv.

We note, in conclusion, that auramine resembles the triphenylmethane dyes studied in having a horizontal $E_1$–pH plot around pH 7 and a high apparent semiquinone stability in this region ($E_1 = 0\cdot049$ v, $K = 38\cdot5$ at pH 7).

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## 619. *The Polarographic Reduction of Pyridine, Quinoline, and Phenazine.*

By R. C. Kaye and H. I. Stonehill.

Pyridine, quinoline, and phenazine, unlike certain bacteriostatic aminoacridines and di- and tri-phenylmethane dyes, do not form apparently highly stable semiquinones on polarographic reduction at pH $\approx$ 7. This is possibly related to their comparatively low bacteriostatic activity. Quinoline and phenazine form relatively unstable semiquinones at pH $\approx$ 7. The pH-dependence of the half-wave potentials of the two one-electron reduction steps for phenazine is similar to that of 1-hydroxyphenazine and of pyocyanine.

We have shown (*J.*, 1951, 27, 2638; and preceding paper) that several bacteriostatic aminoacridines and di- and tri-phenylmethane dyes yield, during polarographic reduction, apparently highly stable semiquinone radicals, which, for the more active of these compounds, are produced at the biologically important pH $\approx$ 7 by uptake of one electron and no proton. In order to substantiate the view that these properties are associated with bacteriostatic activity, we have examined the polarographic reduction of phenazine and (briefly) pyridine and quinoline, three non-bacteriostatic compounds structurally related to acridine.

Shikata and Tachi (*Mem. Coll. Agric. Kyoto*, 1927, **4**, 19) obtained two waves (−1·5 and −1·7 v against the saturated calomel electrode) for pyridine in 0·001N-hydrochloric acid plus 0·1N-potassium chloride, attributed to 6-electron reduction of the ionised and the un-ionised base. Later workers (Adkins and Cox, *J. Amer. Chem. Soc.*, 1938, **60**, 1151; Tompkins and Schmidt, *J. Biol. Chem.*, 1942, **143**, 643; Knobloch, *Coll. Czech. Chem. Comm.*, 1947, **12**, 407; Shchennikova and Korshunov, *J. Phys. Chem. U.S.S.R.*, 1948, **22**, 503; *Chem. Abstr.*, 1948, **42**, 7169) generally agree that one of these waves is a catalytic hydrogen wave, also obtained with pyridine derivatives, and that in buffers no wave is obtained except at pH *ca.* 6—9. For quinoline in alkaline or neutral salt solutions, Pech (*Coll. Czech. Chem. Comm.*, 1934, **6**, 126) obtained two waves of equal height, attributed to successive 2-electron reductions; inadequate buffering and polarogram distortion by adsorption on the dropping mercury electrode probably vitiate these results.

Tachi and Kabai (*J. Electrochem. Assoc. Japan*, 1935, **3**, 250; *Chem. Abstr.*, 1936, **30**, 2500), Adkins and Cox, Knobloch, and Shchennikova and Korshunov (*locc. cit.*) obtained two or more waves for quinoline in various buffered and unbuffered acid, neutral, and alkaline media; some of these waves were ascribed to catalytic hydrogen deposition, and others to adsorption effects. So far as we are aware, no polarographic investigation of phenazine has previously been published, but the reduction of 1-hydroxyphenazine has been studied polarographically by Müller (*Cold Spring Harbor Symp.*, 1939, **7**, 59), and potentiometrically by Michaelis, Hill, and Schubert (*Biochem. Z.*, 1932, **255**, 66).

### Experimental

"AnalaR" pyridine was used without further purification. Quinoline was fractionally distilled, and the fraction of b. p. 236° employed. Phenazine was repeatedly recrystallised from aqueous ethanol; the m. p. of the final product was 169°. All other materials, apparatus, and technique were as previously described (*J.*, 1951, 27). All measurements were at 25°.

### Results and Discussion

*Pyridine.*—For $4 \times 10^{-4}$M-solutions, no pyridine reduction wave was detected in aqueous buffers at pH 1·0, 7·38, and 11·0. In the unbuffered supporting electrolyte used by Shikata and Tachi (*loc. cit.*), one reduction wave (Fig. 1*b*) was obtained, with half-wave potential −1·490 v against the saturated calomel electrode. Applying the Ilkovič equation $\bar{i}_d = 605nD^{\frac{1}{2}}cm^{\frac{2}{3}}t^{\frac{1}{6}}$ (*Coll. Czech. Chem. Comm.*, 1934, **6**, 498), with $\bar{i}_d$ (mean diffusion current) = 3·83 μamp, $c$ (concentration) = 0·4 millimole/l., $m$ (mercury flow rate) = 0·902 mg./sec., $t$ (mercury drop time) = 3·2 sec., $D$ (diffusion coefficient) = $8·6 \times 10^{-6}$ cm.$^2$/sec. (the value given for the geometrically similar molecule benzoquinone by Kolthoff and Orlemann, *J. Amer. Chem. Soc.*, 1941, **63**, 664), we obtain $n$ (number of electrons involved

per molecule) = 4·8. This, together with the value of about 14 mv found for the index potential $E_{\frac{1}{4}}$ (potential difference between the 25% and 50% reduction points), indicates that pyridine forms no semiquinone on reduction.

*Quinoline.*—Well-buffered $4 \times 10^{-4}$M-solutions of pH 2·0—7·4 gave polarograms badly distorted by adsorption effects, which also caused erratic galvanometer oscillations during a single drop life, as with acridine (*J.*, 1951, 27). Inclusion of 50% (by volume) of ethanol in the supporting electrolytes eliminated these effects, as shown by current–time oscillograms (Butler and Kaye, unpublished) similar to those for acridine. However, maxima still remained; these were suppressed by adding 0·04% of gelatin, normal polarograms, typified by Fig. 1*a*, being then obtained.

Applying the Ilkovič equation with $i_d = 1{\cdot}13$ μamp, $c = 0{\cdot}4$ millimole/l., $m = 0{\cdot}902$ mg./sec., $t = 3{\cdot}2$ sec., $D = 3{\cdot}08 \times 10^{-6}$ cm.$^2$/sec., we obtain $n = 2{\cdot}35$. The value of $D$ was obtained from the value $8{\cdot}0 \times 10^{-6}$ for quinaldinic acid in water at 25° (Stock, *J.*, 1944, 427), by deducting 38% from its square root to allow for the change from water to 50% ethanol, by analogy with the results of Gill (Thesis, London, 1947) and of Shreve and Markham (*J. Amer. Chem. Soc.*, 1949, **71**, 2993), as discussed elsewhere (*J.*, 1951, 27). Despite some uncertainty in $D$, it seems that the polarogram is a two-electron wave.

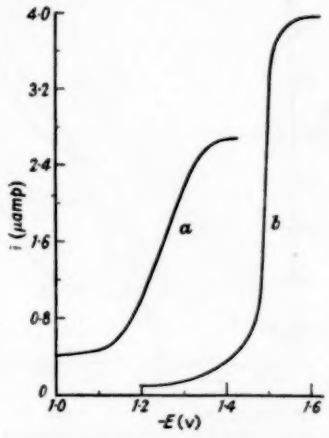



FIG. 1. *Polarograms for: a, quinoline, $4 \times 10^{-4}$M in 50% ethanol,* pH 6·51 *(current scale half of that shown), and b, pyridine, $4 \times 10^{-4}$M in aqueous 0·001N-hydrochloric acid plus 0·1N-potassium chloride.*

From the value $E_{\frac{1}{4}} = 40$ mv at pH 6·51, the semiquinone formation constant $K$ was evaluated by Michaelis's equation $K^{\frac{1}{4}} = 10^{E_{\frac{1}{4}}/0{\cdot}059} - 3 \times 10^{-E_{\frac{1}{4}}/0{\cdot}059}$ (*Ann. N.Y. Acad. Sci.*, 1940, **40**, 39) as 16. Thus the wave consists of two overlapping one-electron steps, involving the intermediate formation of a semiquinone. The latter is apparently much less stable than the semiquinones of acridine and some bacteriostatic aminoacridines and di- and triphenylmethane dyes, for which $K$ ranges from 43·7 to $10^{10}$ in the same solvent and pH region.

It is of interest to compare the above results with those obtained in aqueous media at 25° for quinaldinic acid, 8-hydroxyquinoline, quinoline-8-carboxylic acid (Stock, *J.*, 1944, 427; 1949, 586, 763), and cinchoninic acid (Casimir and Lyons, *J.*, 1950, 783). In all cases, severe distortion of polarograms due to adsorption effects and maxima render the results uncertain, but there is distinct evidence of two single-electron reduction steps at pH $\approx$ 7, the half-wave potential of the first step being independent of pH for only 8-hydroxyquinoline and possibly quinoline-8-carboxylic acid. The index potentials at pH $\approx$ 7 for these four compounds are 0·23, 0·56, 0·80, and 0·60 v respectively, corresponding to $K$ values $7{\cdot}7 \times 10^3$, $2{\cdot}9 \times 10^9$, $3{\cdot}2 \times 10^{13}$, and $1{\cdot}4 \times 10^{10}$ respectively. It is noteworthy that 8-hydroxyquinoline, plasmoquin, and other 8-substituted quinolines display antibacterial activity.

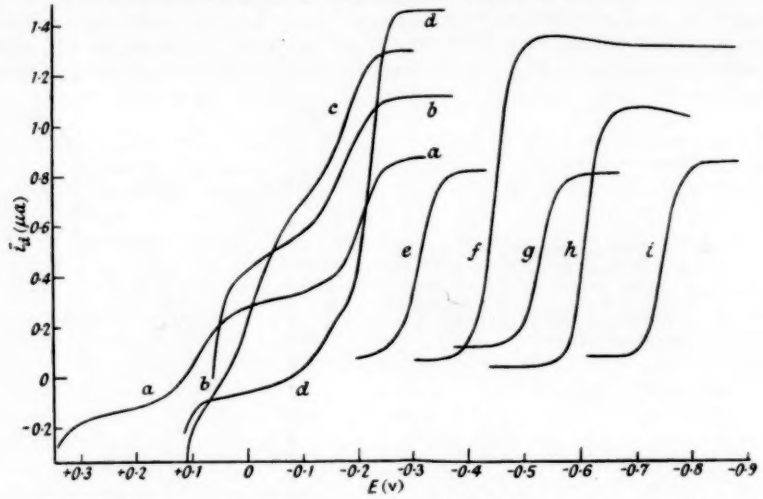
*Phenazine.*—$4 \times 10^{-4}$M-Solutions in aqueous buffers were examined; at pH >2, it was necessary to include 10% by volume of ethanol in the supporting electrolyte because of the low aqueous solubility of the free base.

In strongly acid solutions, reduction occurred in two well-separated steps (Fig. 2), each corresponding to uptake of one electron since the $\bar{i}_d/cm^{\frac{1}{2}}t^{\frac{1}{2}}$ values approximate to those of the closely similar acridine molecule. As the pH increased, these steps moved closer together, and at pH>2 they coalesced into a single two-electron step. In Fig. 3 are plotted against pH (determined for non-alcoholic buffers) values of $K^{\frac{1}{2}}$ calculated from $E_i$ and Michaelis's equation, and also the half-wave potentials $E_1$ and $E_2$ of the first and second steps, calculated from $E_m$ (d.m.e. potential at 50% reduction) by means of the equation

$$E_1 - E_m = E_m - E_2 = 0{\cdot}059 \log K^{\frac{1}{2}}$$

Figs. 2 and 3 show that in acid solutions a stable semiquinone is formed in the first reduction step, but its stability rapidly decreases as pH rises. At pH 3·7, where the $E_1$–pH and $E_2$–pH graphs cross, $K = 1$; at pH $\approx$ 7, $E_i = 17$ mv and $K = 0{\cdot}153$.

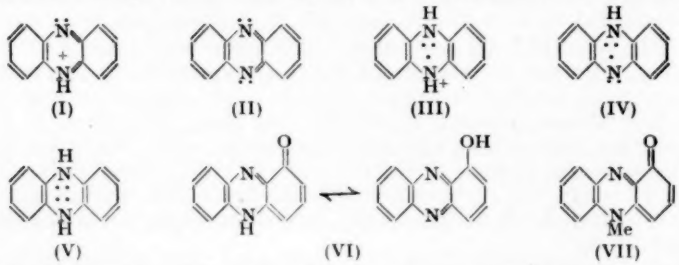
FIG. 2. *Polarograms for phenazine in 10% ethanol at 25°.*




| $10^4$ Concn., M | | pH | $10^4$ Concn., M | | pH |
|---|---|---|---|---|---|
| *a*, | 4 † | 0 (N-$H_2SO_4$) | *f*, | 4 | 7·38 |
| *b*,* | 4 | 1·0 (0·1N-HCl) | *g*, | 2 | 9·07 |
| *c*, | 4 | 1·925 | *h*, | 4 | 11·0 |
| *d*, | 4 | 3·95 | *i*, | 2 | 13·0 |
| *e*, | 2 | 5·58 | | | |

* Anodic wave due to $Cl^-$ swamps first step. † Solvent, $H_2O$; current twice that shown.

Fig. 3 is of practically the same form (at least up to pH 9 or 10) as the $E_1$–pH and $E_2$–pH graphs obtained potentiometrically for 1-hydroxyphenazine (VI) and pyocyanine




(VII) by Michaelis, Hill, and Schubert (*loc. cit.*), who showed unequivocally that semiquinone formation occurred with these substances. Thus a similar phenomenon for

phenazine is confirmed. Furthermore, polarographic values of $E_1$ and $E_2$ for 1-hydroxyphenazine obtained by Müller (*loc. cit.*) fall on the potentiometric $E_1$–pH and $E_2$–pH curves for this compound, when all potentials are referred to the same standard; this shows that polarography is a suitable alternative to potentiometry for investigating redox behaviour.

A familiar procedure (*J.*, 1951, 2638) shows that the two bends in the $E_1$–pH curve (Fig. 3) indicate acid exponents $pK_t = 3{\cdot}2$ for phenazine and $pK_s = 3{\cdot}8$ for its semiquinone. The $pK_s$ value is confirmed approximately by the pH at the sole bend in the $E_2$–pH curve. We assume that phenazine exists in the ionic form $T^+$ (I) at $pH<pK_t$, and as neutral base T (II) at $pH>pK_t$. The slopes of the three linear sections of the $E_1$–pH curve indicate that the numbers of protons involved per molecule reduced in the first step accord with the equations $T^+ + e + H^+ \longrightarrow S^+ (pH<pK_t)$, $T + e + 2H^+ \longrightarrow S^+ (pK_t< pH<pK_s)$ and $T + e + H^+ \longrightarrow S (pH>pK_s)$. Thus the semiquinone exists in the forms $S^+$ (III) and S (IV) at pH respectively below and above $pK_s$. Similarly, for the second

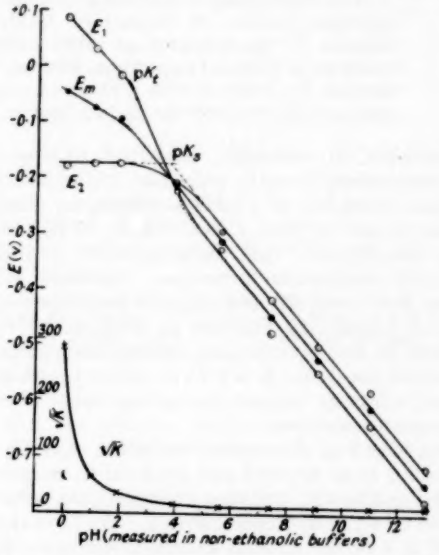



FIG. 3. *Variation with* pH *of* $E_1$, $E_2$, $E_m$, *and* $\sqrt{K}$ *for phenazine,* 4 × $10^{-4}$M *in* 10% *ethanol.*

step we may write $S^+ + e \longrightarrow R(=V)$ for $pH<pK_s$, and $S + e + H^+ \longrightarrow R$ for $pH>pK_s$. By a well-known method (*J.*, 1951, 2638) we then deduce the following equations for the $E_1$–pH and $E_2$–pH curves:

$$E_1 = E_1^0 + 0{\cdot}059 \log \frac{K_s[H^+] + [H^+]^2}{K_t + [H^+]}$$

$$E_2 = E_2^0 + 0{\cdot}059 \log \{[H^+]/([H^+] + K_s)\}$$

in which $E_1^0$ and $E_2^0$ are the values of $E_1$ and $E_2$ respectively at pH = 0.

Species $S^+$ is stabilised by equivalent resonance, in which, *e.g.*, the odd electron may reside on either nitrogen atom. No equivalent resonance is possible for S, which explains why semiquinone stability becomes very small at $pH>pK_s$. The semiquinone obtained by oxidising *p*-phenylenediamine behaves similarly (Michaelis, Schubert, and Granick, *J. Amer. Chem. Soc.*, 1939, **61**, 1981).

The present results show that non-bacteriostatic pyridine, quinoline and phenazine, unlike several antibacterial aminoacridines and di- and tri-phenylmethane dyes, do not form apparently highly stable semiquinones during reduction at pH ≈ 7.

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## 620. *The Polarographic Reduction of Some Natural and Artificial Hydrogen-carriers in Bacterial Enzyme Systems.*

By R. C. Kaye and H. I. Stonehill.

Cozymase, riboflavin, methylene-blue, Capri-blue, and cresyl-blue in aqueous solution at pH 7·38, and Nile-blue in 50% ethanol at pH 8·29, undergo polarographic reduction at 25° in two overlapping one-electron stages, with the intermediate formation of semiquinones whose stability is low compared with that of the semiquinones of several antibacterial aminoacridines and di- and tri-phenylmethane dyes. The polarographic results regarding semiquinone formation with cozymase and methylene-blue agree with those of earlier potentiometric studies, justifying the use of the polarograph for investigations of semiquinone formation.

The results suggest that the above and other substances which may act as hydrogen carriers or respiratory catalysts in bacterial enzyme systems function by participation of labile semiquinone radicals in radical-chain reactions in bacterial respiration, whereas the antibacterials mentioned above function by reaction with a chain-carrying radical to form a highly stable semiquinone, whereby the chain is broken.

Riboflavin, a naturally occurring hydrogen carrier in bacterial enzyme systems, undergoes potentiometric reduction in two overlapping one-electron steps, with the intermediate formation of a labile semiquinone (Stern, *Biochem. J.*, 1934, **28**, 949; Michaelis, Schubert, and Smythe, *J. Biol. Chem.*, 1936, **116**, 587; Haas, *Biochem. Z.*, 1937, **290**, 291). Haas also reported that the attachment of riboflavin to its protein carrier in enzymes favoured semiquinone formation. Similarly, Michaelis, Schubert, and Granick (*J. Amer. Chem. Soc.*, 1940, **62**, 204) showed potentiometrically that thionine and methylene-blue, both of which may function as artificial hydrogen carriers in bacterial respiration, are reduced in two overlapping one-electron steps, the intermediate semiquinones having formation constants $K \approx 0{\cdot}05$ at pH $\approx 7$, although $K$ for thionine increased considerably in acid solutions because the semiquinone, after addition of a proton, was stabilised by equivalent resonance.

The object of the present work was to verify that the polarographic method yielded $K$ values for some natural and artificial hydrogen carriers comparable with those obtained potentiometrically, and then to investigate polarographically the stability of semiquinones formed by other hydrogen carriers. We have therefore studied the polarographic reduction at pH $\approx 7$ of the natural hydrogen carriers cozymase and riboflavin, and the artificial hydrogen carriers methylene-blue and oxazines Capri-blue (Colour Index No. 876), Nile-blue (C.I. No. 913), and cresyl-blue (C.I. No. 877). We were unaware of the previous polarographic investigation of methylene-blue and riboflavin by Brdička (*Z. Elektrochem.*, 1941, **47**, 721; 1942, **48**, 278, 686) until after this work was completed. However, Brdička was concerned with adsorption effects at the dropping mercury electrode, and not greatly with $K$ values.

### Experimental

Cozymase (from Schwarz Laboratories Inc., New York) was of 60% purity; the impurities did not apparently give a polarographic reduction wave. Riboflavin and methylene-blue were of B.P. purity. Nile-blue was recrystallised from dilute hydrochloric acid, and cresyl-blue from ethanol. Capri-blue was too soluble to be recrystallised from water or ethanol; it was purified by being salted out from aqueous solution with sodium chloride and dried at low temperature, whereafter the dye was extracted from any sodium chloride present by absolute ethanol, the extract was evaporated, and the residue dried at low temperature (to avoid tar formation). The purification of the last three dyes was followed polarographically, the wave-height for constant weight concentration increasing progressively.

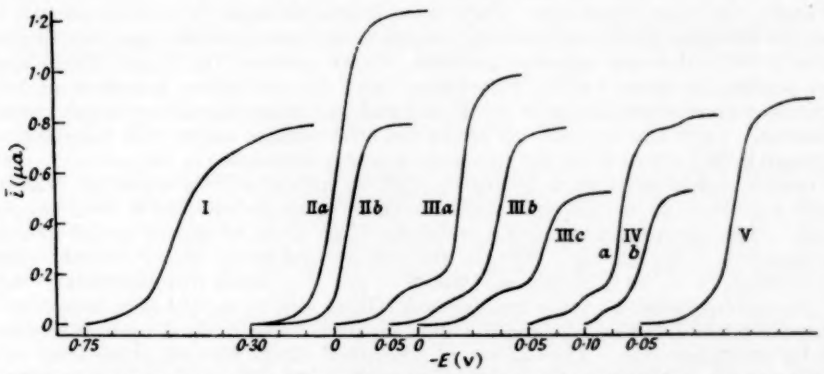
Apparatus, technique, and other experimental details were as described previously (*J.*, 1951, 27). Potentials were measured at 25° against the saturated calomel electrode.

## Results

*Cozymase.*—At concentration $c$ = 0·2 millimole/l. (after allowance for the 40% of impurity) in aqueous phosphate buffer of pH 7·38, a single reduction wave (curve I) was obtained, with half-wave potential $E_m$ = −0·927 v, wave-height $\bar{i}_d$ = 0·4 μa (corr. for residual current), and index potential $E_i$ = 0·0345 v (difference between the dropping mercury electrode potentials at 50% and 25% or 75% reduction). The slight abnormality near the top of the wave might be due to adsorption at the mercury electrode, as with 2 : 8-diaminoacridine (*J.*, 1951, 2638), or to reduction of some impurity; since supply of cozymase was limited, this was not further investigated. Comparison of $\bar{i}_d/cm^{\frac{2}{3}}t^{\frac{1}{6}}$ = 605 $nD^{\frac{1}{2}}$ (Ilkovič, *Coll. Czech. Chem. Comm.*, 1934, **6**, 498) with the value for acridine (*J.*, 1951, 27) suggests that, despite the fact that the diffusion coefficient $D$ of cozymase is unknown, $n$ (the number of electrons involved per molecule reduced) = 2 (in the above equation, $m$ = mg. of mercury flowing per sec., and $t$ = drop-time in sec.). On this assumption, substitution of the above $E_i$ value in Michaelis's equation (*Ann. N.Y. Acad. Sci.*, 1940, **40**, 39)

$$K^{\frac{1}{2}} = 10^{E_i/0\cdot0591} \quad -3 \times 10^{-E_i/0\cdot0591}$$

gives $K$ = 9·3. Thus reduction occurs in two overlapping one-electron steps, with the intermediate formation of a semiquinone whose stability is relatively low compared with the semiquinones of the acridines and di- and tri-phenylmethane dyes listed in the Table. The existence of semiquinone is confirmed by the appearance of a transient yellow colour, attributed to monohydrocozymase, lasting not longer than 30 seconds, during the reduction of cozymase with sodium dithionite in boiling water (Gutcho and Stewart, *Anal. Chem.*, 1948, **20**, 1185).




*Polarograms for* (I) *cozymase*, ca. 2 × $10^{-4}$M, pH 7·38 *in water* (*current scale half that shown*); (II) *riboflavine*, 4 × $10^{-4}$M, (*a*) pH 7·38 *in water*, (*b*) pH 8·29 *in 50% ethanol*; (III) *methylene-blue*, (*a*) 4 × $10^{-4}$M, pH 7·38 *in water*, (*b*) 4 × $10^{-4}$M, pH 8·29 *in 50% ethanol*, (*c*) 2 × $10^{-4}$M, pH 7·38 *in water*; (IV) *Capri-blue*, *ca.* 4 × $10^{-4}$M, (*a*) pH 7·38 *in water*, (*b*) pH 7·32 *in 50% ethanol*; (V) *cresyl-blue*, 4 × $10^{-4}$M, pH 7·38 *in water*. *Voltage scale intervals* 0·05v.

*Riboflavin.*—A 4 × $10^{-4}$M-solution in aqueous phosphate buffer at pH 7·38 gave a single wave (curve II*a*), with $E_m$ = −0·460 v and $\bar{i}_d$ = 1·34 μa. It being assumed, in the absence of data, that $D$ is approximately the same as for acridine, comparison of $\bar{i}_d/c\ m^{\frac{2}{3}}t^{\frac{1}{6}}$ values gives $n$ = 2 (nearest integer). Hence, since $E_i$ = 0·023 v, $K$ = 1·49. Thus again we have two overlapping one-electron steps and a labile semiquinone. The inclusion of 50% by volume of ethanol in the buffer decreased $\bar{i}_d$ and raised the apparent pH to 8·29, but caused little change in $E_i$ or $K$ (curve II*b*).

*Methylene-blue.*—This was examined over the concentration range 2—8 × $10^{-4}$M. In aqueous phosphate buffer at pH 7·38, a single wave (curves III*a*, *c*) was obtained, with an anomalous adsorption fore-wave. Over the potential range covered by the fore-wave, erratic galvanometer oscillations were observed. The fore-wave height was indepehdent

of, and the total wave-height was proportional to, concentration, confirming Brdička's results (*loc. cit.*). The inclusion of 50% of ethanol only partly suppressed the fore-wave, and decreased the total wave-height by about 25%. Even 55% of ethanol did not completely remove the fore-wave, and higher ethanol contents induced crystallisation of buffer salts. With $i_d = 1{\cdot}0$ μa for $c = 0{\cdot}4$ millimole/l. in water, $D = 4{\cdot}20 \times 10^{-6}$ cm.$^2$/sec. (Brdička, *loc. cit.*), $m = 0{\cdot}902$ and $t = 4{\cdot}0$, the Ilkovič equation gives $n = 1{\cdot}71$, *i.e.*, 2. In aqueous solution at pH 7·38, $E_i = 0{\cdot}016$ v, whence $K = 0{\cdot}065$, in fair agreement with the potentiometric value 0·05 (Michaelis, Schubert, and Granick, *loc. cit.*). In 50% ethanol at apparent pH 8·29, $E_i = 0{\cdot}022$ v, whence $K = 1{\cdot}16$, showing that ethanol increases semiquinone stability, as found by Burstein and Davidson (*Trans. Electrochem. Soc.*, 1941, **80**, 175) for certain anthraquinone derivatives.

*Capri-, Nile-, and cresyl-blue.*—These were studied in aqueous solution at pH 7·38, except for Nile-blue, which was sparingly soluble in the aqueous buffer, and was therefore, studied in 50% ethanol at apparent pH 8·29. Capri- (curves IV*a*, *b*) and Nile-blue gave waves similar to those for methylene-blue, with an adsorption fore-wave of height independent of concentration up to $10^{-3}$M, and with accompanying abnormal galvanometer oscillations. Capri-blue also gave an adsorption after-wave in water; this was completely suppressed in 50% ethanol, but the fore-wave was only diminished in this solvent. Cresyl-blue gave a normal polarogram (curve V), with no indication of adsorption. Values of $i_d/cm^{\frac{2}{3}}t^{\frac{1}{6}}$ for these three dyes were similar to those of methylene-blue in the appropriate solvent. If we assume a corresponding similarity of $D$ values because of similar molecular structures, $n = 2$ in each case. For the three dyes respectively, $E_m = -0{\cdot}218$, $-0{\cdot}400$, and $-0{\cdot}222$ v, $E_i = 0{\cdot}0165$, 0·0200, and 0·0200 v, and $K = 0{\cdot}104$, 0·643 and 0·643. Apart from the more negative $E_m$ value for Nile-blue, largely due to the pH shift caused by the presence of ethanol, the $E_m$, $E_i$, and $K$ values for these three oxazine dyes are similar to those for methylene-blue. The absence of adsorption effects for cresyl-blue alone of the four dyes is presumably due to the presence in it of the hydrophilic amino-group. Although Nile-blue also has such a group, its effect is more than offset by the extra aromatic hydrophobic ring.

## Discussion

The agreement between our polarographic and earlier potentiometric determinations of the stability ($K$) of the semiquinones of riboflavin and methylene-blue justifies the use of the polarograph to determine the extent of semiquinone formation. We may thus with confidence deduce from our results that the electroreduction and semiquinone formation characteristics of cozymase, Capri-blue, Nile-blue, and cresyl-blue are similar to those of riboflavin and methylene-blue.

In the accompanying Table are listed the $K$ values of several compounds studied polarographically in this and previous work (*J.*, 1951, 27, 2638; 1952, 3231), and also that of pyocyanine obtained potentiometrically (Michaelis, Hill, and Schubert, *loc. cit.*). The values refer mostly to 25° and pH $\approx 7$ in water; although some values are for 50% ethanol as solvent and its presence is known usually to increase $K$, this will not appreciably affect the relative order of magnitude of the $K$ values.

Their $K$ values divide the compounds into two main groups, nos. 1—11 (large $K$) and nos. 13—20 (small $K$). Compounds 1—11 are respiratory anticatalysts in bacterial systems and mostly powerful antibacterials, especially where reduction to semiquinone at pH $\approx 7$ requires no proton uptake. Nos. 13—20 are natural and artificial respiratory catalysts or hydrogen carriers, although no. 17 sometimes inhibits bacterial growth at high concentrations. The intermediate position of quinoline (no. 12) is interesting, since some of its 8-substituted derivatives, *e.g.*, 8-hydroxyquinoline, are strong antibacterials.

In a theory of the mechanism of respiratory catalysts and anticatalysts based on these results (Kaye, *J. Pharm. Pharmacol.*, 1950, **2**, 902), it is supposed that bacterial respiration is a radical-chain reaction. Substances which can provide relatively labile semiquinone radicals may participate in the reaction chain, thus acting as catalysts (cf. Parravano, *J. Amer. Chem. Soc.*, 1951, **73**, 183). Compounds which readily form highly stable semiquinones on reduction, especially if no proton is taken up along with the electron (thus

(*Apparent*) *Semiquinone formation constants* (K) *at* 25°.

| No. | Compound | Solvent | pH | *K* | |
|---|---|---|---|---|---|
| 1 | Acridine | 50% Ethanol | 7 | $10^{8}$ | Respiratory anticatalysts and antibacterials |
| 2 | 1-Aminoacridine | ,, | 7 | $10^{10}$ | |
| 3 | 2- ,, | ,, | 7 | $10^{10}$ | |
| 4 | 3- ,, | ,, | 7 | $10^{8}$ | |
| 5 | 4- ,, | ,, | 7 | $10^{10}$ | |
| 6 | 5- ,, | ,, | 7 | $10^{7}$ | |
| 7 | 2 : 7-Diaminoacridine | ,, | 7 | $10^{7}$ | |
| 8 | 2 : 8- ,, | ,, | 7 | $10^{8}$ | |
| 9 | Crystal-violet | ,, | 7 | $10^{9\cdot75}$ | |
| 10 | Brilliant-green | ,, | 7 | $10^{4\cdot75}$ | |
| 11 | Auramine | ,, | 7 | 38·5 | |
| 12 | Quinoline | ,, | 6·51 | 16 | |
| 13 | Cozymase | Water | 7·38 | 9·3 | Natural respiratory catalysts |
| 14 | Riboflavin | ,, | 7·38 | 1·49 | |
| 15 | Pyocyanine (30°) | ,, | 7·38 | 0·062 | |
| 16 | Phenazine | ,, | 7·38 | 0·153 | Artificial respiratory catalysts |
| 17 | Methylene-blue | ,, | 7·38 | 0·065 | |
| 18 | Capri-blue | ,, | 7·38 | 0·104 | |
| 19 | Nile-blue | 50% Ethanol | 8·29 | 0·643 | |
| 20 | Cresyl-blue | Water | 7·38 | 0·643 | |

minimising entropy decrease), act as chain-breakers by reacting with a labile chain-radical to form a stable semiquinone; they are therefore anticatalysts or antibacterials.

Objection to this theory of drug action may be raised because of the very negative reduction potentials required by these compounds. Thus for compounds nos. 6—11 the half-wave potentials for the first reduction step at pH 7, referred to the normal hydrogen electrode standard, are −0·984, −0·594, −0·761, −0·546, −0·424, and −0·926 v, respectively; $E_{\frac{1}{2}}$ for cozymase at pH 7·38 is −0·686 v on the same scale. Such highly negative potentials are unlikely to be encountered in bacterial cells, although no reliable measurements of bacterial cell potentials exist. Comparison of the last figure with the potentiometric values −0·270 v (Green and Dewan, *Biochem. J.*, 1937, **31**, 1069) and −0·290 v (Stephenson, " Bacterial Metabolism," Longmans Green and Co., 1939) suggests a possible answer to the objection, *viz.*, an overvoltage effect at the dropping mercury electrode renders polarographic potentials far more negative than potentiometric values. Another possibility is that attachment of semiquinone to the enzyme protein (or nucleic acid; cf. Oster, *Trans. Faraday Soc.*, 1951, **47**, 660) may facilitate reduction to semiquinone at less negative potentials than at the dropping mercury electrode, just as adsorption of methylene blue semiquinone on the electrode permits reduction of the dye at less negative potentials than are required in the absence of adsorption (Brdička, *loc. cit.*).

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## 621. N-*Arylglycosylamines.*

By S. Bayne and W. H. Holms.

In the *N*-chlorophenyl- and *N*-tolyl-glucosylamines, and in the *N*-carboxyphenylglucosylamines the *o*-isomers are more stable in air at room temperature than are the *m*- and *p*-isomers. By using reduction of 2 : 6-dichlorophenolindophenol as an index of the transformation it has been shown that, in weakly acidic solutions, the *o*-isomers are less readily converted into the corresponding *iso*glucosamines than are the *m*- and *p*-isomers.

Preparation of the glucosylamine from *p*-toluidine by Sorokin (*J. pr. Chem.*, 1888, **37**, 291) was the first example of the condensation of a sugar with a substituted aniline; and although Irvine and Gilmour (*J.*, 1908, 1429; 1909, 1545) and Irvine and Hynd (*J.*, 1911, 161) prepared a number of compounds of this nature, no comparative study of the

various isomers was possible until Hanaoka (*J. Biochem., Japan*, 1938, **28**, 109) completed the toluidine series and Sannie and Lapin (*Bull. Soc. chim.*, 1948, 892) completed the carboxyaniline series.

Kuhn and Weygand (*Ber.*, 1937, **70**, 769) and Weygand (*Ber.*, 1939, **72**, 1663; 1940, **73**, 1248, 1259) demonstrated the transformation of glycosylamines into *iso*glycosamines by acid and suggested an Amadori rearrangement as its mechanism. *iso*Glycosamines are well-defined crystalline compounds which may be regarded as ketose derivatives, exhibit mutarotation, probably possess a cyclic structure, and, unlike the glycosylamines, reduce methylene-blue and 2 : 6-dichlorophenolindophenol (Kuhn and Birkofer, *Ber.*, 1939, **71**, 621).

During an investigation in this laboratory into the utilisation of aromatic amines as catalysts in the preparation of osazones, glycosylamines were prepared by various methods (Irvine and Gilmour, and Weygand, *locc. cit.*; cf. Kuhn and Ströbele, *Ber.*, 1937, **70**, 773), and some were converted into the isomeric *iso*glycosamines. Many of the glycosylamines and *iso*glycosamines, especially if washed with peroxidised ether, rapidly decomposed to black tarry materials. When standardised methods of preparation and purification were adopted, with precautions to avoid the incorporation of unchanged amine in the crystalline products, certain regularities in behaviour were noticed. The times for formation of *N*-tolyl-D-glucosylamine under standard conditions, as assessed by attainment of homogeneity in the reaction mixture, were *o*- 40, *m*- 25, and *p*- 20 minutes: the glucosylamine is formed more slowly as the substituent methyl group approaches the amino-group. The same regularity was noted for the decomposition: at various rates of decomposition, the rapidity of "browning" was $p > m > o$. This held in a general way also for the glucosylamines from *o*-, *m*-, and *p*-carboxyaniline, and *o*-, *m*-, and *p*-chloroaniline, and the galactosylamines from *o*- and *p*-toluidine. *N*-Phenyl-D-glucosylamine was the least stable of the glucosylamines prepared (cf. Honeyman and Tatchell, *J.*, 1950, 967). The *iso*glucosamines of *o*- and *p*-toluidine, although decomposing much less readily than the corresponding glucosylamines, show the same differential effect of *o*- and *p*-substitution. It thus appears that any substitution in the aromatic ring of certain series of glycosylamines or *iso*glycosamines stabilises the products and that the effect increases with the position of the substituent in the order $p < m < o$.

Unsuccessful attempts were made to measure the rate of decomposition of the glucosylamines by colorimetric estimation of the brown colour produced, according to the method employed by Wolfrom, Cavalieri, and Cavalieri (*J. Amer. Chem. Soc.*, 1947, **69**, 2411) in their studies on the interaction of amino-compounds and sugars. Since an acid environment may lead to hydrolysis (Hanaoka, *loc. cit.*), decomposition, or isomeric rearrangement, depending on such variables as temperature, concentration, type of acid used, and time of reaction, it seems possible that the decomposition of the glycosylamines may proceed *via* aldose residues produced by hydrolysis or *via* the *iso*glycosamines (1):

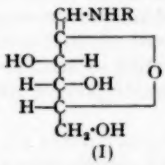
$$\text{Glycosylamine} \longrightarrow \textit{iso}\text{Glycosamine} \longrightarrow \text{Tar} \quad . \quad . \quad . \quad . \quad (1)$$

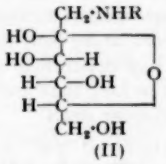
The latter hypothesis is supported by the observation that samples of glycosylamine which have undergone partial decomposition show the reducing properties characteristic of *iso*glycosamine rather than of the free sugar. On the other hand, pure *iso*glycosamines themselves decompose more slowly in acid conditions than the parent glycosylamines and an alternative route (2) for the decomposition process is possible:

$$\text{Glycosylamine} \longrightarrow \begin{matrix} \text{Unsaturated} \\ \text{intermediates} \end{matrix} \begin{matrix} \nearrow \text{Tar} \\ \rightleftharpoons \textit{iso}\text{Glycosamine} \end{matrix} \quad . \quad . \quad . \quad (2)$$

Some support for the second mechanism is afforded by Gottschalk and Partridge's observations (*Nature*, 1950, **165**, 684) on the effect of acid on aliphatic and aromatic glycosylamines. They suggest that (I) may be an intermediate in this decomposition to humin substances and 5-hydroxymethylfurfuraldehyde, being formed by loss of water from (III) which was suggested by Weygand (*Ber.*, 1940, **73**, 1259) as an intermediate in the transformation of glucosylamine to *iso*glucosamine, or from the furanose form (II) of

the *iso*glucosamine. Under mild acid conditions fructofuranose undergoes the same change (Haworth and Jones, *J.*, 1944, 667).


(I)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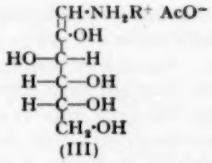

(III)

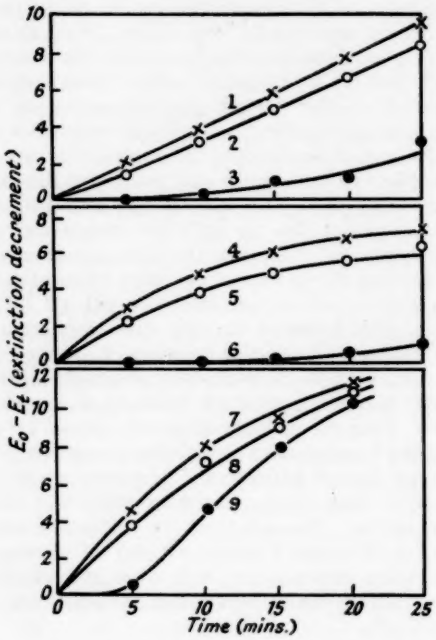
*iso*Glycosamines were prepared by Weygand (*Ber.*, 1940, **73**, 1259) in good yield by heating glycosylamines from several hexoses and pentoses in very dilute hydrochloric or acetic acid. Berger and Lee (*J. Org. Chem.*, 1946, **11**, 76) demonstrated that the *N*-phenyl- and the *N*-*o*-4-xylyl-ribosylamines did not undergo an Amadori rearrangement under these conditions and Berger, Solmssen, Leonard, Wenis, and Lee (*J. Org. Chem.*, 1946, **11**, 91) obtained crystalline ribose by hydrolysing *N*-phenyl-D-ribosylamine in hot dilute acetic acid, removing the amine by steam-distillation or combination with benzaldehyde; however, Kuhn and Birkofer (*loc. cit.*) had noted that certain glycosylamines do not undergo the Amadori rearrangement to *iso*glycosamines and it seems likely that both the structure of the sugar and the nature of the parent amine may determine the type of decomposition. Kuhn *et al.* showed that, unlike the free sugars and the glycosylamines, the *iso*glycosamines reduce 2 : 6-dichlorophenolindophenol (cf. also Berger and Lee, *loc. cit.*). From preliminary experiments it was apparent that at 100° the change was too rapid for convenient measurement by this reagent, and that when the process was carried out in dilute acetic acid the pH changed, altering the colour of the dye. Comparisons were finally made at or near room temperature in an acetate buffer of pH 4·7. This technique, which assumes that the reduction of indophenol parallels the production of *iso*glycosamine, was satisfactory for isomerisations of *N*-tolyl- (curves 1—3) and *N*-chlorophenyl-glucosylamines (curves 4—6), but with *N*-carboxyphenylglucosylamines it gave inconsistent results. The pH of freshly made 1% aqueous solutions of the three *N*-carboxyphenylglucosylamines was 3·7—4·0; thus the irregularities were caused by the ionisation of the free carboxyl groups, catalysing isomerisation to the *iso*glucosamines, the effect varying with the length of time that had elapsed between the preparation and use of the solutions. Preliminary experiments with these glucosylamines showed that other decomposition reactions occurred in alkaline media. The solutions were therefore made up with 0·1N-sodium hydroxide to give ultimate pH values between 5·8 and 6·0; isomerisation was then minimised, alkaline decomposition was avoided, and, when the solutions were used soon after preparation, the carboxyaniline series behaved in the same way as the toluidine and chloroaniline series (curves 7—9).

It is evident that the *o*-isomer, in each series, isomerises more slowly than the *m*- or *p*-isomer—cf. the differing ease of decomposition discussed above. Hanaoka (*loc. cit.*) studied the hydrolysis of these series by mineral acid (see below), and there is an analogous study of the enzyme efficiency (Wertigkeit) of sweet-almond β-glucosidase in the hydrolysis of substituted β-phenylglucosides (Helferich and Phillipp, *Annalen*, 1934, **514**, 228; Helferich and Lutzmann, *ibid.*, 1939, **537**, 11). In the latter substitution by methyl or carboxyl groups increased the rate of hydrolysis in the order $p < m < o$, in direct contradiction to our results for the glucosylamines; glycosides, however, show fundamental chemical differences from glycosylamines and, although the normal forms of certain of the latter are considered to be β-glycopyranosylamines by Butler, Smith, and Stacey (*J.*, 1949, 3371) (cf. also Howard, Kenner, Lythgoe, and Todd, *J.*, 1946, 855) and by Ellis and Honeyman (*Nature*, 1951, **167**, 239) who have, by a different procedure isolated a new isomer of *N*-*p*-tolyl-D-glucosylamine (cf. Irvine and Gilmour, *loc. cit.*), they are not hydrolysed by β-glucosidase (Pigman, *J. Res. Nat. Bur. Stand.*, 1943, **30**, 257).

Hanaoka (*loc. cit.*; *J. Biochem., Japan*, 1940, **31**, 95) followed the hydrolysis of glucosylamine to glucose and free amine (by sulphuric acid in aqueous methyl alcohol) by measuring the rotation of the mixture, assuming that the only optically active substances

present in the solution are glucosylamine as an equilibrium mixture and free glucose; however, as has been shown above, the solution probably contains also the corresponding *iso*glucosamines; in fact Weygand (*Ber.*, 1940, **73**, 1259) has obtained good yields of *iso*glucosamine by employing hydrochloric acid as a catalyst in the Amadori rearrangement. The *iso*glucosamines are in general lævorotatory and the polarimetric method is therefore suspect. Hanaoka's confirmatory method depending on the reduction of alkaline copper solutions does not take cognisance of the fact that any *iso*glucosamine present will effect the reduction even more readily than glucose itself.

Hanaoka showed that in the *N*-tolyl- and *N*-chlorophenyl-D-glucosylamines the rate of "hydrolysis" increases in the order *o*- < *m*- < *p*-, which is directly comparable with our results. In the *N*-carboxyphenyl-D-glucosylamine series, he found that the *o*-derivative was more readily "hydrolysed" than the *p*-derivative. This is at variance with our




N-*Tolyl*-D-*glucosylamines*: 1, p-; 2, m-; 3, o-.

N-*Chlorophenyl*-D-*glucosylamines*: 4, p-; 5, m-; 6, o-.

N-*Carboxyphenyl*-D-*glucosylanmines*: 7, p-; 8, m-; 9, o-.

findings both for decomposition and for isomerisation as measured by the production of indophenol-reducing material. The disagreement may be explicable in terms of the rotational difference between the *iso*glucosamines which are formed under these conditions, and the glucose which was assumed by Hanaoka to be the end-product of the reaction.

## Experimental

Amines employed were purified by distillation or by crystallisation from 95% ethanol. M. p.s are corrected. For analysis all compounds were dried *in vacuo* over phosphoric oxide at 60°. Analyses for carbon and hydrogen are by Drs. Weiler and Strauss, Oxford.

N-*Tolyl*-D-*glucosylamines*.—The method of preparation was essentially that of Weygand (*Ber.*, 1939, **72**, 1663), and gave *N*-*o*-tolyl-D-glucosylamine hemihydrate (61·5%), m. p. 95—96°, $[\alpha]_D^{20}$ −79·0° ⟶ −50·0° (*c*, 1·00 in methanol), N-m-*tolyl*-D-*glucosylamine hemihydrate* (74·5%), m. p. 106—107°, $[\alpha]_D^{20}$ −97·5° ⟶ −49·5° (*c*, 1·00 in methanol) (Found: C, 56·2; H, 7·2; N, 5·1. $C_{13}H_{19}O_5N,\frac{1}{2}H_2O$ requires C, 56·1; H, 7·2; N, 5·0%) (Hanaoka, *J. Biochem., Japan*, 1938, **28**, 109, gives m. p. 117°, $[\alpha]_D$ −102·9° ⟶ −50·3°), and *N*-*p*-tolyl-D-glucosylamine hemihydrate (75·8%), m. p. 114—115°, $[\alpha]_D^{20}$ −98·5° ⟶ −46·5° (*c*, 1·00 in methanol).

*N-o*-Tolyl-D-glucosylamine prepared as above and kept in a desiccator over sulphuric acid did not discolour during 46 days. *N-p*-Tolyl-D-glucosylamine under identical conditions displayed appreciable " browning " after 28 days.

N-*Carboxyphenyl*-D-*glucosylamines*.—Irvine and Gilmour's method (*loc. cit.*) gave *N-o*-carboxyphenyl-D-glucosylamine monohydrate (74·2%), m. p. 131—132° (decomp.), $[\alpha]_D^{18}$ $+65{\cdot}0° \longrightarrow -9{\cdot}5°$ (after 24 hours) (*c*, 0·50 in 50% ethanol).

Sannie and Lapin's method (*loc. cit.*) gave *N-m*-carboxyphenyl-D-glucosylamine monohydrate (77·8%), m. p. 122—123° (decomp.), $[\alpha]_D$ $+18{\cdot}0° \longrightarrow -41{\cdot}5°$ (after 24 hours) (*c*, 0·50 in 50% ethanol), and *N-p*-carboxyphenyl-D-glucoside monohydrate (70·8%), m. p. 132—133° (decomp.), $[\alpha]_D^{18}$ $-104°$ to $-105° \longrightarrow -48{\cdot}5°$ (after 24 hours) (*c*, 0·50 in 50% ethanol). Hanaoka (*J. Biochem., Japan*, 1940, **31**, 95) gives m. p. 127°, $[\alpha]_D$ $-112{\cdot}0° \longrightarrow -51{\cdot}5°$ (in methanol), and Dansi (*Farm. Sci. tecn.*, 1947, **2**, 195) m. p. 134—135°, $[\alpha]_D$ $-194° \longrightarrow +30°$ (in methanol) for the latter.

It was noted by Sannie and Lapin (*loc. cit.*), following the earlier observations of Irvine and Gilmour (*loc. cit.*) on *N-o*-carboxyphenyl-D-glucosylamine, that the mutarotations of the *N*-carboxyphenylglucosylamines depend on the conditions of preparation. In the present investigation the equilibrium values of [α] were not readily obtainable and solutions of these glucosylamines developed a powerful reducing action towards methylene-blue and indophenol after a few hours at room temperature. The complexity of this process appears to explain the variable results reported for the optical rotations of these compounds which have been otherwise clearly defined.

N-*Chlorophenyl*-D-*glucosylamines*.—Irvine and Gilmour's method (*loc. cit.*) gave N-o-*chlorophenyl*-D-*glucosylamine hemihydrate* (70·0%), m. p. 139—140°, $[\alpha]_D^{17}$ $-72{\cdot}0°$ (*c*, 0·50 in methanol) (Found: C, 48·4; H, 6·1; N, 4·7. $C_{12}H_{16}O_5NCl,\frac{1}{2}H_2O$ requires C, 48·2; H, 5·7; N, 4·7%) (Hanaoka, *J. Biochem., Japan*, 1940, **31**, 95, gives m. p. 137°, $[\alpha]_D$ $-50{\cdot}5° \longrightarrow +5{\cdot}0°$), N-m-*chlorophenyl*-D-*glucosylamine hemihydrate* (67·1%), m. p. 116—117°, $[\alpha]_D^{17}$ $-121{\cdot}0°$ (*c*, 0·5 in methanol) (Found: C, 48·5; H, 5·7; N, 5·0%), and N-p-*chlorophenyl*-D-*glucosylamine hemihydrate* (66·7%), m. p. 120—122°, $[\alpha]_D^{17}$ $-30°$ (*c*, 0·5 in methanol) (Found: C, 48·4; H, 5·9; N, 4·7%) (Hanaoka, *loc. cit.*, gives m. p. 126°, $[\alpha]_D$ $-40° \longrightarrow -22{\cdot}5°$). Hanaoka makes no mention of water of crystallisation.

The *N*-chlorophenylglucosylamines did not exhibit measurable mutarotation within a period of 96 hours. After a further 72 hours at room temperature $[\alpha]_D$ had risen by about 2°. The addition of a trace of acid (Hanaoka, *loc. cit.*) initiated a further rise which did not reach an equilibrium value and was accompanied by browning and development of indophenol-reducing properties by the methanolic solution. Kuhn and Birkofer (*loc. cit.*) noted the absence of rotational changes in *N-o*-nitrophenylglycosylamines and the reasons for this were examined by Howard, Kenner, Lythgoe, and Todd (*loc. cit.*).

N-*Phenyl*-D-*glucosylamine*.—When prepared by Weygand's method (*Ber.*, 1939, **72**, 1663), (59%), this had m. p. 134—136°, $[\alpha]_D^{20}$ $+10{\cdot}5° \longrightarrow -52{\cdot}2°$ (*c*, 0·50 in methanol).

N-*Tolylgalactosylamines*.—The method of preparation was essentially that used for the *N*-tolylglucosylamines. The mixture containing *o*-toluidine became homogeneous in 4 minutes and that containing *p*-toluidine in 2 minutes.

N-o-*Tolyl*-D-*galactosylamine hemihydrate* (58·5%) had m. p. 114—116°, $[\alpha]_D^{16}$ $-41{\cdot}0° \longrightarrow -18{\cdot}0°$ (*c*, 1·00 in methanol) (Found: C, 56·3; H, 7·5; N, 5·1. $C_{13}H_{19}O_5N,\frac{1}{2}H_2O$ requires C, 56·1; H, 7·2; N, 5·0%); the *hemihydrate* of the *p*-isomer (72·5%) had m. p. 160—162°, $[\alpha]_D^{16}$ $-56{\cdot}0 \longrightarrow -23{\cdot}5°$ (*c*, 1·00 in methanol) (Found: C, 56·3; H, 7·4; N, 5·4%) (cf. Ellis and Honeyman, *J.*, 1952, 1490).

The *N*-tolylgalactosylamines did not reduce neutral or alkaline 2 : 6-dichlorophenolindophenol but a few minutes after the addition of dilute acetic acid developed reducing properties towards that reagent with decolorisation in either acid or alkaline solution.

N-o- *and* N-p-*Tolyl*-D-iso*glucosamines*.—Weygand's method (*Ber.*, 1940, **73**, 1259) gave o- (62%), m. p. 126—128°, $[\alpha]_D^{17}$ $-19{\cdot}0° \longrightarrow -4{\cdot}0°$ (*c*, 0·50 in methanol) (Found: C, 58·0; H, 7·2; N, 5·8. $C_{13}H_{19}O_5N$ requires C, 58·0; H, 7·1; N, 5·2%) and *p*-tolyl-D-*iso*glucosamine (84%), m. p. 152—154°, $[\alpha]_D^{17}$ $-23{\cdot}0° \longrightarrow -10{\cdot}0°$ (*c*, 0·50 in methanol). Kuhn and Weygand (*loc. cit.*) give m. p. 150—152°, $[\alpha]_D$ $-63{\cdot}8° \longrightarrow -22{\cdot}4°$. Both showed the characteristic reducing properties described by Kuhn and Birkofer (*loc. cit.*).

*Assessment of Glycosylamine and* iso*Glucosamine Decomposition*.—A thin layer of pure glycosylamine or *iso*glucosamine on a watch-glass was exposed to an atmosphere saturated with water vapour and acetic acid at about 25°. The time taken for each sample to reach an arbitrarily chosen degree of browning was noted. Simultaneous measurements (see Table)

were carried out on the members of an *o*-, *m*-, *p*-series but no attempt was made to compare the glycosylamines from different amines.

| | *o* | *m* | *p* |
|---|---|---|---|
| *Glucosylamines* | | | |
| Tolyl ..... | 40 mins. | 12 mins. | 8 mins. |
| Carboxyphenyl ... | 75 mins. | 20 mins. | 15 mins. |
| Chlorophenyl ..... | 20 mins. | 9 mins. | 6 mins. |

| | *o* | *p* |
|---|---|---|
| *Galactosylamines* | | |
| Tolyl ..... | 30 mins. | 10 mins. |
| iso*Glucosamines* | | |
| Tolyl ..... | 16 hrs. | 4 hrs. |

*Measurement of Rate of Isomerisation to* iso*Glucosamine.*—The *iso*glucosamine formed in an acid medium was determined colorimetrically by its reduction of 2 : 6-dichlorophenolindophenol. To a 1% aqueous solution of the glucosylamine (2 ml.) was added 0·01% aqueous 2 : 6-dichlorophenolindophenol (2 ml.). Acetate buffer (pH 4·7; 5 ml.) was added and the gradual disappearance of the red colour at room temperature was measured in a photoelectric colorimeter with an Ilford 621 violet filter, readings being taken at intervals of 5 minutes. Simultaneous observations were carried out on the *N*-tolyl-, *N*-chlorophenyl-, and *N*-carboxyphenylglucosylamines. In the case of the last-named sufficient 0·1N-sodium hydroxide was added to the 1% aqueous solution during its preparation to adjust the pH to 6·0 and prevent autoisomerisation before addition of the acetate buffer and dye.

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## 622. *Reductions with Aluminium Alkoxides. Part III.* The Kinetics of the Racemization of Optically Active Alkoxides by their Corresponding Ketones.*

By L. M. Jackman and A. Killen Macbeth.

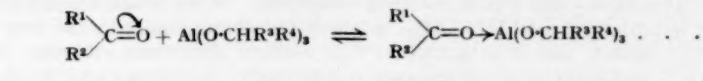
The existing evidence for a postulated mechanism of the reduction of carbonyl compounds by aluminium alkoxides is reviewed. The racemization of aluminium (+)-1-methylpropoxide and aluminium (+)-1-methylheptyloxide by butan-2-one and octan-2-one respectively has been followed polarimetrically under various conditions of concentration and temperature. The presence of *cyclo*hexylamine has been shown to retard the reaction. The kinetic interpretation of these results is discussed in the light of the postulated mechanism.

In view of the high specificity of the reaction, the mechanism of the Meerwein–Ponndorf–Verley reduction represents a peculiar problem. Various mechanisms have been proposed for it, the most feasible involving the initial co-ordination between the alkoxide and ketone, followed by the intramolecular transfer of hydrogen through a cyclic transition state. This type of mechanism, first proposed by Meerwein (*J. pr. Chem.*, 1936, **147**, 211), has since been adopted by other workers (Woodward, Wendler, and Brutschy, *J. Amer. Chem. Soc.*, 1945, **67**, 1425; Dewar, "Electronic Theory of Organic Chemistry," Oxford, 1949, p. 136; Jackman and Mills, *Nature*, 1949, **164**, 789). Hammett ("Physical Organic Chemistry," McGraw-Hill Book Co., New York, 1940, p. 352) also suggested co-ordination as the first step, but then assumed the hydrogen transfer to proceed intermolecularly. The two steps in the mechanism involving the cyclic transition state are represented as (1) and (2). Although there is little direct evidence for this mechanism, it is found adequately to explain all known facts concerning the reduction, and some of the more important of these are now summarized, the facts being dealt with in two classes, according to whether they are consequent upon the first or the second stage of the reaction.

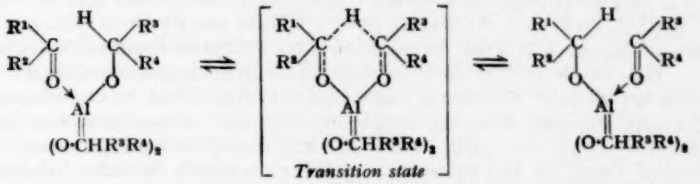
The high specificity of the aluminium atom may be considered under the first heading. Although the alkoxides of aluminium are reducing agents *par excellence*, the alkoxides of other elements are also capable of functioning in such reversible reduction systems.

* Part II, *J.*, 1949, 2646.

Magnesium ethoxide (Meerwein and Schmidt, *Annalen*, 1925, **444**, 221), halogenomagnesium alkoxides (Meerwein and Schmidt, *loc. cit.*; Gomberg and Bachmann, *J. Amer. Chem. Soc.*, 1930, **52**, 4967; Shankland and Gomberg, *ibid.*, p. 4973; Bachmann and Kloetzel, *ibid.*, 1937, **59**, 2210), and boron alkoxides (Wuyts and Duquesne, *Bull. Soc. chim. Belg.*, 1939, **48**, 77; Kuivila, Slack, and Siiteri, *J. Amer. Chem. Soc.*, 1951, **73**, 123) have all been shown to bring about reduction but are all inferior to the aluminium derivatives in ease and scope of reduction. These elements all possess *p*-orbitals available for co-ordination. It is to be expected that the Group III elements might exhibit a greater tendency to co-ordinate,



. . . . (1)




. (2)

thus explaining why aluminium is more effective than magnesium. Similarly, within Group III itself it might be expected that the first member, boron, in view of its greater co-ordinating ability, would be a more effective reducing agent than aluminium, whereas in fact the reverse is true and reductions of aldehydes with *iso*propyl borate require many hours' heating at 150°. The alkyls and fluoride of boron are strongly co-ordinating, but the alkoxides probably show no such tendency because of a highly effective overlap of the oxygen $2p_z$ orbital with the vacant $2p_z$ orbital of boron. Mulliken (*ibid.*, 1950, **72**, 4493) has recently calculated the overlap integrals for various heteropolar bonds involving first- and second-row elements, and it would appear that $p_\pi$-orbital overlap is less effective for elements of the second row. Although the type of bond considered above has not been dealt with by Mulliken, it is reasonable to suppose that they also follow this rule. The fact then that the $3p_\pi$–$2p_\pi$ bond in aluminium alkoxides is less effective than in the boron compounds would permit the formation of stronger $3p_\sigma$–$2p_\sigma$ bonds with a carbonyl oxygen atom. This concept of $\pi$-bonding, which has been termed "back co-ordination," is reflected in the absence of association of boric esters in solution (Sidgwick, "Chemical Elements and Their Compounds," Oxford, 1950, Vol. I, p. 387). It is significant that alkoxides of aluminium usually possess association factors of 3—5 in benzene solution (Robinson and Peake, *J. Phys. Chem.*, 1935, **39**, 1125; Ulich and Nespital, *Z. phys. Chem.*, 1933, **165**, 294). The marked difference in co-ordinating power and the relative $\pi$-bond formation of the oxy-compounds of aluminium and boron may be further gauged by results of certain *X*-ray crystallographic studies. The crystal of boric acid is found to consist of planes of molecules held together by hydrogen bonds (Zachariasen, *Z. Krist.*, 1934, **88**, 150) and the B–O distance is 1·35 Å, compared with 1·54 Å, the sum of the two covalent radii. The B–O distance corresponds to approximately one-third double-bond character in each of the three bonds and implies that boron has a complete octet. In analogous aluminium compounds, hydrargillite (Megaw, *ibid.*, 1934, **87**, 185) and diaspore (Ewing, *J. Chem. Phys.*, 1935, **3**, 203), the aluminium is found to be in the six-covalent state and the Al–O distance corresponds, within the somewhat large limits of experimental error, to the sum of the covalent radii. Electron-diffraction studies (Bauer and Beach, *J. Amer. Chem. Soc.*, 1941, **63**, 1394) show a similar shortening of the B–O distance in methyl borate, but unfortunately, values are not available for aluminium alkoxides. A similar difference between the compounds of boron and aluminium is well illustrated by the dipole moments of the halides in benzene solution (*Trans. Faraday Soc.*, 1934, **30**, Appendix). Boron trichloride has a zero dipole moment, indicating a planar configuration as a result of back co-ordination (Pauling, "Nature of the Chemical Bond," Cornell Univ. Press, New York, 1945, p. 238), but aluminium bromide is apparently non-planar, as it has a dipole

moment of 4·89 D. Further evidence of the non-co-ordination of boron esters has been summarised by Yabroff, Branch, and Almquist (*J. Amer. Chem. Soc.*, 1933, **55**, 2935). Almost certainly, back co-ordination exists in the aluminium alkoxides, but to a lesser extent than in boron analogues. The difference in reducing power of the boron and aluminium compounds may then arise from their relative ability to co-ordinate with the carbonyl compounds.

In the light of the above discussion it would seem most unlikely that stable addition compounds with aluminium alkoxides would exist, other than in solution, except, perhaps, in the most favourable cases and under ideal conditions. A few examples can be quoted. Salts of the hypothetical acid $HAl(OR)_4$, in which the aluminium is in the four-covalent state, are well known, and it is significant that sodium aluminium ethoxide (Meerwein *et al.*, *loc. cit.*), and magnesium aluminium *iso*propoxide (Kulpinsky and Nord, *J. Org. Chem.*, 1943, **8**, 256) are devoid of reducing power. There are, of course, the various well-known chelate compounds which may be regarded as co-ordination compounds of phenoxides or alkoxides. The complexes with acetylacetone and 8-hydroxyquinoline are quite stable. These examples are all most favourable cases, and it is found that no co-ordination compounds of the type resulting from the simple union of two neutral molecules have been isolated. Chelinzev (*Bull. Soc. chim.*, 1924, **35**, 741) claims to have produced evidence, based on thermal data, for the existence of such compounds between iodomagnesium alkoxides and alcohols and ketones. It thus appears that aluminium alkoxides owe their specificity to their ability to co-ordinate with carbonyl compounds to a degree *sufficient* to permit the reaction to proceed at a measurable speed. Further evidence based on physical measurements will, however, be sought to confirm this point. In the Meerwein–Ponndorf reduction, then, the amphoteric aluminium alkoxides may be regarded as functioning as acids. With the realization of this fact, the formal analogy between this reaction and the base-catalysed Cannizzaro reaction disappears, and a similarity in mechanisms is no longer to be expected (cf. Verley, *ibid.*, 1927, **39**, 797; Waters, " The Chemistry of Free Radicals," 2nd edn., Oxford, 1948, p. 192). In addition to the alkoxides already discussed Meerwein (*loc. cit.*) has reported that benzaldehyde can be reduced in varying yields by the ethoxides of zirconium(IV), tin(IV), titanium(IV), antimony(V), and iron(III), and the ease of reduction appears to be in that order. All are inferior to aluminium ethoxide, although the zirconium derivative may be classed as a " good " reducing reagent. That these elements can exercise higher valencies as their ethoxides has been shown by Meerwein and Bersin (*Annalen*, 1929, **476**, 113), who prepared their " Alkoxosalze " with the alkoxides of Group I and II elements; *e.g.*, the compounds $[Zr(OEt)_6]HNa$, $[Ti(OBu)_6]HK$, and $[Sn(OEt)_6]K_2$ were prepared. Furthermore, the halides of zirconium, stannic tin, and titanium are known to form addition compounds with one or two molecules of ethers or ketones (Scagliarini and Tartarini, *Atti Accad. Lincei*, 1926, **4**, 318; Evard, *Compt. rend.*, 1933, **196**, 2007; Jantsch, *J. pr. Chem.*, 1927, **115**, 7).

Concerning the second step of the reduction, it has been stated elsewhere (Jackman and Mills, *loc. cit.*) that the path through the cyclic transition state is to be favoured because of the possibility of resonance in such a system, with an equivalent lowering of the activation energy. It was pointed out at the same time that models indicated the transition state to be favourable on steric grounds. There are certain experimental facts which also favour this mechanism. First, the fact that αβ-double bonds are not reduced was mentioned as a possible consequence of the cyclic transition state, as 1 : 4-addition would require a sterically less favourable eight-membered ring. This view has recently been confirmed by the work of Lutz and Gillespie (*J. Amer. Chem. Soc.*, 1950, **72**, 344). Perhaps more significant are the stereochemical results of the reductions of certain alkyl*cyclo*hexanones with various alkoxides (Jackman, Macbeth, and Mills, *J.*, 1949, 2641), which receive interpretation in the light of this mechanism. Similarly, the achievement of asymmetric syntheses in this reaction (Doering and Young, *J. Amer. Chem. Soc.*, 1950, **72**, 631; Jackman, Mills, and Shannon, *ibid.*, p. 4814), and the configurations of the predominent enantiomers, are consistent with the theory.*

* McGowan (*Chem. and Ind.*, 1951, 601) has provided a very elegant confirmation of the postulated cyclic transition state.

In view of the nature of the above evidence, a kinetic study of the reaction seemed desirable in order to provide more precise confirmation. No investigation of the kinetics of the reduction has been reported, and there are certain problems which complicate such work. The reduction process may be considered as the establishment of an equilibrium between two oxidation–reduction systems (cf. Baker and Adkins, *ibid.*, 1940, **62**, 3305), and to follow the chemical change it is necessary, in the general case, to be able to estimate *one* alcohol or *one* ketone in the presence of the other alcohol or other ketone. The use of a light-absorption technique was given some consideration, as it was thought that with fluorenone–fluorenol as one of the systems the progress of the reaction might be followed. This idea was abandoned in preference to that reported below, for the kinetics of the equilibrium reaction were complicated by the formation of mixed alkoxides and the necessity of dealing with two competing reactions. The light-absorption method, however, may be capable of development, and could be useful as it offers a wider scope than the present method. The kinetic procedure now described consists of the measurement of the rate of racemization of an aluminium derivative of an optically active alcohol by the *corresponding* ketone. This method greatly simplifies the kinetics, as the possibility of the formation of mixed alkoxides no longer exists, and the actual rate process under consideration goes to completion unaffected by an opposing reaction. The chief disadvantage of this method is that it is confined to alkoxides of the few readily accessible alcohols which have their asymmetric centres at the hydroxylated carbon atoms and cannot undergo epimerization. Of these the active butan-2-ols and octan-2-ols are used here. The resolutions of the two alcohols (Ingersoll, " Organic Reactions," Vol. II, John Wiley, 1944, pp. 400, 403) give reasonably high yields and can be carried out quickly and on a fairly large scale. (−)-Aluminium 1-methylpropoxide (but-2-oxide) is prepared by the action of aluminium on the (+)-alcohol. This method is not available for aluminium 1-methylheptyloxide (octyl-2-oxide) as this alkoxide cannot readily be distilled nor can the crude product be purified by chromatography. The desired compound was eventually obtained in a pure state by the use of an alcohol-exchange reaction (cf. Baker and Lynn, *J. Amer. Chem. Soc.*, 1949, **71**, 1399) between the octanol and aluminium *tert.*-butoxide in toluene. The *tert.*-butanol may be removed, as it is formed, as its azeotrope with toluene. This alkoxide has the opposite sign of rotation to its parent alcohol. Attempts to carry out the exchange without a solvent failed to go to completion, and the resulting mixed alkoxide, when aluminium *iso*propoxide is used, exhibits an interesting mutarotation when its solution in benzene is treated with octan-2-one.

## Experimental

*Methylcyclohexane.*—Commercial samples were shaken with one-tenth their volume of oleum for 2 hours. The hydrocarbon was decanted and washed with sodium hydroxide solution (5%) and then with water, until neutral to litmus. The product was dried ($MgSO_4$), fractionated over sodium, and stored over this metal. Spectroscopic analysis showed the complete absence of aromatic compounds.

*Butan-2-one.*—Commercial ethyl methyl ketone was purified by conversion into its bisulphite compound and fractionation of the regenerated ketone. The last traces of moisture were removed by prolonged storage over anhydrous copper sulphate.

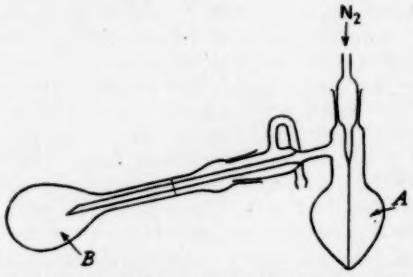
*Octan-2-one.*—A solution of sulphuric acid (96 ml.; *d* 1·83) in water (200 ml.) was added dropwise to a mechanically stirred mixture of octan-2-ol (130 g.), powdered potassium dichromate (195 g.), and water (800 ml.). The acid was added at such a rate as to keep the mixture at 60°. After the addition of the acid was complete, stirring was continued until the temperature fell to 25°. The oily layer was separated, and washed with water, sodium hydroxide solution (5%), and finally with water. The crude octan-2-one was added to a solution of semicarbazide (105 g.) and crystalline sodium acetate (130 g.) in water (500 ml.). Ethanol (350 ml.) was added to dissolve the ketone, and an immediate formation of the semicarbazone took place. The reaction mixture was set aside in a refrigerator overnight, and the crystalline material was filtered off and washed with 50% ethanol (100 ml.). After one recrystallisation from 50% ethanol the semicarbazone (132 g.) melted sharply at 122°. A solution of the semicarbazone in 5% sulphuric acid (3 equiv.) was steam-distilled. The distillate was extracted twice with ether and the extract dried ($MgSO_4$). After removal of the solvent the octan-2-one was distilled. The product

(88 g., 69% calc. on octan-2-ol), b. p. 64°/15 mm., was stored over anhydrous copper sulphate until required.

(+)-*Butan-2-ol.*—The resolution was carried out by Ingersoll's method (*loc. cit.*). The hydrolysis of the active hydrogen phthalate was modified slightly. A solution of (+)-1-methylpropyl hydrogen phthalate (65 g.) in 10% aqueous sodium hydroxide (290 ml.) was fractionated through a 60-cm. column (packed with beads) under a reflux ratio of 4 : 1. The water–butanol azeotrope, b. p. 86·5°/762 mm., distilled first and the fractionation was continued until the temperature of the distillate reached 100°. The distillate was shaken with anhydrous potassium carbonate (15 g.), and the aqueous layer removed. The alcohol was refluxed and distilled over freshly prepared calcium oxide. The last traces were removed from the calcium oxide by momentarily evacuating the system. The active alcohol (17·0 g.) had $[\alpha]_D^{22\cdot2}$ +10·65° homogenous. Residues from the resolution yielded samples of (−)-butan-2-ol with $\alpha_D$ as high as −4·18°, which were useful for preliminary experiments.

*Aluminium* (+)-1-*Methylpropoxide* (*But-2-oxide*).—Aluminium foil (3·65 g.) was treated with (+)-butan-2-ol (35 g.) and a few mg. of mercuric chloride. The mixture was refluxed until the foil had completely dissolved. The excess of alcohol was removed under reduced pressure in a stream of dry nitrogen. The alkoxide was distilled under reduced pressure into the flask *A* (Fig. 1) and from thence redistilled (b. p. 146—154°/0·55 mm.) into an accurately weighed 100-ml. graduated flask *B*. The use of this technique greatly minimized the risk of hydrolysis during the subsequent handling of the product. The aluminium (+)-but-2-oxide (32·246 g.) was then dissolved in methyl*cyclo*hexane and made up to 100 ml.

FIG. 1.




(+)- *and* (−)-*Octan-2-ol.*—Both optically active forms were obtained by Ingersoll's method (*loc. cit.*) and dried by refluxing over one-tenth of their weight of fresh calcium oxide.

*Aluminium* (±)-1-*Methylheptyloxide* (*Octyl-2-oxide*).—(*a*) Aluminium foil (1·0 g.) was treated with dry (±)-octan-2-ol (15 g.) and a few mg. of mercuric chloride. The mixture was heated in a heating mantle and a vigorous reaction ensured near the b. p. of the alcohol. Heating was continued until all the aluminium had dissolved (½ hour). The excess of alcohol was removed on a water-pump (b. p. 87°/17 mm.). Attempts to distil the alkoxide by ordinary techniques led to decomposition, but it could be distilled in a " short-path " still at $10^{-2}$ mm./240—250° (bath temp.). This method was slow and unsuitable for large-scale preparations.

The crude alkoxide prepared as above was probably substantially pure except for the presence of suspended impurities of undissolved metals. Long periods of centrifuging failed to remove these impurities, which must have been colloidal. Neutral alumina was prepared by permitting redistilled aluminium *iso*propoxide to hydrolyse spontaneously in a desiccator over water. The traces of moisture were removed at 700° in an electric furnace. This alumina was used in an attempted chromatography of the alkoxide in benzene, but the colloidal material was eluted as readily as the alkoxide.

(*b*) Aluminium *iso*propoxide (11·0 g.) was distilled under reduced pressure in the apparatus illustrated in Fig. 1. To the redistilled aluminium *iso*propoxide (9·80 g.) in the standard flask *B* was added dry (±)-octan-2-ol (24·69 g.). A dried boiling chip (0·05 g.) was added, the flask was fitted for distillation, and then heated in a bath at 120°, whereupon a liquid (6 ml.), b. p. 88—92°, distilled. The distillation was now continued at 9 mm. (bath temp. 130°), and a stream of dry nitrogen drawn through the residue. Finally, the system was evacuated to $10^{-2}$ mm. In this way a colourless viscous product (17·75 g.) was obtained. This compound was assayed for aluminium by the 8-hydroxyquinoline method (Found : Al, 7·35. Calc. for $C_{24}H_{51}O_3Al$ : Al, 6·5%). It was concluded that some *iso*propoxy-residues still remained.

(*c*) Carefully purified aluminium *tert.*-butoxide (5·0 g.) was dissolved in a mixture of toluene (200 ml.) and (±)-octan-2-ol (9·0 g.). The solution was fractionated through a 30-cm. column packed with single-turn Fenske glass helices and fitted with a reflux divider. With a reflux ratio of 10 : 1, a toluene–*tert.*-butanol azeotrope, b. p. 81·5°/757 mm., was slowly removed. A further quantity (100 ml.) of toluene was taken off. The residue was cooled and rapidly transferred to a dry centrifuge cone and traces of turbidity were removed by centrifuging. Finally, the solvent and excess of octanol were removed by distillation in a current of dry nitrogen at 150°/0·1 mm. (bath temp.) (4 hours). The clear viscous liquid thus obtained was (±)-*aluminium* 1-*methylheptyloxide* (*octyl-2-oxide*) (Found : Al, 6·4. $C_{24}H_{51}O_3Al$ requires Al, 6·5%).

*Aluminium* (+)- *and* (−)-1-*Methylheptyloxide*.—An optically active mixed alkoxide was prepared by method (*b*). Samples of pure (+)- and (−)-alkoxides were obtained by method (*c*). It was noted that (+)-octan-2-ol, $[\alpha]_D^{15}$ +8·10° (homogeneous), yielded (−)-aluminium 1-methylheptyloxide, $[\alpha]_D^{20\cdot0}$ −8·52 (*c*, 12·8 in methyl*cyclo*hexane). Slight hydrolysis could therefore have a marked effect on the rotation of the alkoxide.

cyclo*Hexylamine*.—Commercial samples were fractionated over sodium through a short column of single-turn helices; b. p. 134°.

*The Kinetic Method*.—The polarimetric measurements were carried out on a Hilger Barfit polarimeter fitted with a 2-dm. jacketed brass tube of 9—10 ml. capacity with a side tube to facilitate filling. Sodium-*D* lines were used for all measurements. Water from a thermostat was circulated through the jacket of the tube, and the temperature of the effluent could be held to ±0·02°.

The ketone solutions were dispensed from a 5-ml. automatic microburette fitted with a 250-ml. reservoir and carefully protected from the atmosphere by "anhydrone" tubes. Samples of the alkoxide solutions were transferred in vacuum pipettes. All apparatus was dried before use by washing them with anhydrous ether, and evaporating the ether in a stream of dry air. It was found that the solutions could be introduced into the polarimeter tube within 1 minute and that equilibrium temperature was reached within 3 min., so the first reading could be made approximately 4 min. after the admixing.

*Results*.—(The standard deviation has been employed as the precision index throughout. Unless otherwise stated, methyl*cyclo*hexane is the solvent used for all experiments.)

For each experiment, the racemization process was found to obey, within certain limits, the general first-order equation

$$k = (1/2t) \ln \alpha_0/\alpha_t \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

where $\alpha_0$ and $\alpha_t$ are rotations at zero time and time $t$ (seconds), respectively. The coefficient $\frac{1}{2}$ takes into account the fact that the racemization is complete when one-half of the alkoxide molecules have undergone inversion (cf. Hughes *et al.*, *J.*, 1935, 1526). For a process of this type it is possible to select any point during the reaction as an arbitrary zero. Table 1 gives

TABLE 1. *The variation of the rate constant with time for the racemization of* (+)-*aluminium* 1-*methylpropoxide* (0·6545M) *by butan-2-one* (0·360M).

| $t$ (min.) | $\alpha^{30}$ | $10^5k'$ | $10^5k''$ | $t$ (min.) | $\alpha^{30}$ | $10^5k'$ | $10^5k''$ | $t$ (min.) | $\alpha^{30}$ | $10^5k'$ | $10^5k''$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | +2·40° | — | — | 51 | +2·26° | 1·43 | — | 124 | +1·96° | 1·56 | 1·60 |
| 11 [a] | 2·42 | — | — | 57 [b] | 2·235 | 1·44 | — | 139 | 1·90 | 1·59 | 1·65 |
| 17 | 2·41 | 0·55 | — | 64 | 2·20 | 1·50 | 1·90 | 154 | 1·84 | 1·59 | 1·67 |
| 21 | 2·40 | 0·68 | — | 73 | 2·16 | 1·53 | 1·79 | 167 | 1·79 | 1·61 | 1·69 |
| 26 | 2·38 | 0·93 | — | 82 | 2·13 | 1·49 | 1·60 | 195 | 1·69 | 1·63 | 1·69 |
| 31 | 2·35 | 1·23 | — | 89 | 2·10 | 1·52 | 1·62 | 211 | 1·65 | 1·59 | 1·65 |
| 37 | 2·33 | 1·21 | — | 100 | 2·05 | 1·55 | 1·67 | 242 | 1·555 | 1·59 | 1·66 |
| 41 | 2·31 | 1·30 | — | 106 | 2·025 | 1·57 | 1·68 | 269 | 1·49 | 1·59 | 1·58 |
| 46 | 2·285 | 1·36 | — | 115 | 1·99 | 1·57 | 1·67 | 298 | 1·41 | 1·59 | 1·58 |

[a] Denotes the zero for $k'$. [b] Denotes the zero for $k''$.

the result of a characteristic experiment on the racemization of (+)-aluminium 1-methylpropoxide by butan-2-one; $k'$ and $k''$ are rate constants calculated by taking zero values at the beginning and at 6·9% completion respectively.

Fig. 2 shows the plot of log $\alpha_0/\alpha_t$ against time for the first 13% of the reaction, and it is seen that a linear relationship only exists after an initial period of inconsistency. For this reason the rate constants for the various experiments have been calculated by selecting, as zero values, times at which the reaction has passed this initial period. As the accuracy of the rate constants depends, in part, on the precision of the zero measurements, three such values have been obtained for each experiment. In order to avoid the measurement of very small differences, reasonable

intervals of time were allowed to elapse between the " zero " readings and the first $t$ reading. Ten such $t$ readings were made and then an arithmetic mean of all 30 rate constants thus calculated was computed and accepted as the rate constant for the particular experiment. After the reactions had proceeded past 50% completion, downward drifts in the rate constants were invariably observed. Readings were therefore confined to the first half of the reaction. The drift is almost certainly a consequence of a mesityl oxide type of condensation which is well known as a side reaction of the Meerwein–Ponndorf reduction (cf. Wayne and Adkins, *J. Amer. Chem. Soc.*, 1940, **62**, 3401). It is significant that the drift was found to occur earlier at higher ketone concentrations, which is consistent with the probable dependence of the rate of condensation on the *square* of the ketone concentration.

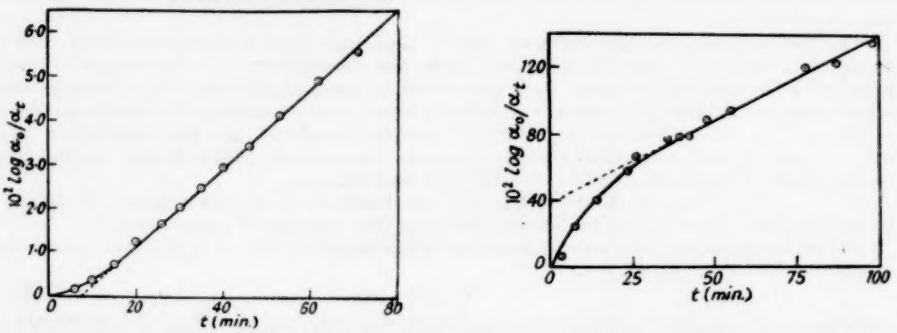
Table 2 indicates that the rate of racemization is dependent on the concentration of ketone and the rate equation now becomes

$$k_2 = (1/[\mathrm{B}]t) \ln \alpha_0/\alpha_t \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

where [B] is the ketone concentration.

Similar results have been obtained for the system (−)-aluminium 1-methylheptyloxide–octan-2-one, although in this case the nature of the initial stage of the reaction differs from that of the (+)-aluminium 1-methylpropoxide experiments. Fig. 3 shows the plot of log $\alpha_0/\alpha_t$ for the early part of a characteristic experiment. It is seen here that the rate of racemization appears to be greatest at the commencement of the reaction. Blank experiments, without

FIG. 2.




ketone, showed no variation of rotation with time, and even the most vigorous drying of the ketone failed to alter the observed result. The results recorded in Table 3 show equation (4) to be obeyed for the racemization of constant concentrations of aluminium 1-methylheptyloxide. In both Figs. 2 and 3 only a part of that actual fraction of the reaction for which first-order kinetics was obeyed has been plotted. For the experiments with 1-methyl-propoxide and -heptyloxide the initial period of inconsistency extended over the first 7—9% and 15—20%, respectively, and in each case the rate equations have been based on at least 40% of the reaction subsequent to the initial period.

TABLE 2. *The effect of ketone concentration.*

(+)-Aluminium 1-methylpropoxide, 0·6545M; 30·00°.

| Ketone molarity | 0·180 | 0·240 | 0·300 | 0·360 | 0·420 |
|---|---|---|---|---|---|
| $10^5 k_2$ (l. g.-mol.$^{-1}$ sec.$^{-1}$) | 4·61 ± 0·10 | 4·59 ± 0·06 | 4·53 ± 0·11 | 4·60 ± 0·06 | 4·53 ± 0·11 |

TABLE 3. *The effect of ketone concentration.*

(+)-Aluminium 1-methylheptyloxide, 0·227M; 30·0°.

| Ketone molarity | 0·120 | 0·160 | 0·200 | 0·400 |
|---|---|---|---|---|
| $10^5 k_2$ (l. g.-mol.$^{-1}$ sec.$^{-1}$) | 8·90 ± 0·51 | 8·90 ± 0·42 | 9·25 ± 0·37 | 9·05 ± 0·68 |

Experiments with varying alkoxide concentration indicate that, in spite of the nature of equation (4), the absolute alkoxide concentration has a marked effect on the rate of racemization of (+)-aluminium 1-methylheptyloxide. The results obtained fit the general equation (5) over the limited range examined: [A] is the total alkoxide concentration. The closeness of fit

$$k' = ([\mathrm{A}]/[\mathrm{B}]t) \ln \alpha_0/\alpha_t \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

of this equation may be assessed from rate constants (Table 4) obtained at various concentrations of aluminium 1-methylheptyloxide. The theoretical significance of this unexpected observation that the kinetics of the racemization are of first order in ketone, but independent of alkoxide

TABLE 4. *The effect of alkoxide concentration.*

Octan-2-one concentration, 0·200M; 30·0°.

| Alkoxide molarity | 0·154 | 0·227 | 0·270 | 0·386 |
|---|---|---|---|---|
| $10^5k'$, sec.$^{-1}$ | 2·20 ± 0·15 | 2·10 ± 0·08 | 2·16 ± 0·11 | 2·11 ± 0·08 |

concentration, will be discussed later. Meanwhile, it is obvious that the Arrhenius equations must be based on this new equation (5). Similar results have been observed with (±)-aluminium 1-methylpropoxide in which the values 5·09 and 4·59 for $10^5k_2$ (equation 4) were obtained for two experiments under identical conditions, with the exception of the alkoxide molarities, which were 0·627M and 0·654M, respectively.

Tables 5 (*a*) and (*b*) give the values of the rate constants at various temperatures for the racemization of (+)-aluminium 1-methylpropoxide and (−)-aluminium 1-methylheptyloxide, respectively.

TABLE 5. *The effect of temperature.*

(*a*) (+)-Aluminium 1-methylpropoxide, 0·6275M; butan-2-one, 0·240M.

| Temp. | 20·15° | 22·60° | 25·00° | 27·00° | 30·00° | 33·75° |
|---|---|---|---|---|---|---|
| $10^5k'$ (sec.$^{-1}$) | 1·20 ± 0·04 | 1·49 ± 0·09 | 1·95 ± 0·06 | 2·45 ± 0·03 | 3·20 ± 0·07 | 5·00 ± 0·11 |

(*b*) Aluminium 1-methylheptyloxide, 0·227M; octan-2-one, 0·220M.

| Temp. | 22·10° | 25·00° | 26·95° | 30·00° | 31·90° |
|---|---|---|---|---|---|
| $10^5k'$ (sec.$^{-1}$) | 1·15 ± 0·08 | 1·44 ± 0·07 | 1·78 ± 0·16 | 2·10 ± 0·10 | 2·56 ± 0·26 |
| | 1·21 ± 0·04 | 1·40 ± 0·08 | 1·79 ± 0·11 | — | 2·16 ± 0·13 |
| Grand means | 1·20 ± 0·04 | 1·42 ± 0·11 | 1·79 ± 0·10 | 2·10 ± 0·10 | 2·22 ± 0·13 |

Calculations of the Arrhenius parameters from the data in Table 5 have been carried out by the method of " least squares " (Worthing and Geffner, " Treatment of Experimental Data," John Wiley, 1943, p. 240). In the treatment of the data in Table 5(*b*) the grand means were assigned weights equivalent to the inverse squares of their standard deviations and these weights were then included in the " least squares " calculations. The results are shown in equations (6) and (7) for the methylpropoxide and the methylheptyloxide, respectively :

$$k' = 1{\cdot}15(\pm 0{\cdot}05) \times 10^9 e^{-18,800(\pm 900)/RT} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

$$k' = 8{\cdot}22(\pm 2{\cdot}41) \times 10^4 e^{13,400(\pm 2,900)/RT} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

The larger errors in the case of (−)-aluminium 1-methylheptyloxide are a result of the numerically small observed rotations. The approximate entropies of activation at 30·0° have been calculated from equations (6), (7), and (8) (Glasstone, Laidler, and Eyring, " Theory of Rate Processes," McGraw-Hill Book Co., 1941, p. 417), by assuming a transmission coefficient of unity. These values are −18 and −38 E.U. for (+)-1-methylpropoxide and (−)-1-methylheptyloxide, respectively.

$$k = e(kT/h)e^{\Delta S^{\ddagger}/R}e^{-\Delta E^{\ddagger}/RT} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

The addition of *cyclo*hexylamine was shown to have a marked effect on the rate of racemization of (−)-aluminium 1-methylheptyloxide. At the same time, a reduction in the initial rotation of the alkoxide solutions was observed. Table 6 summarizes these results.

TABLE 6. *Effect of the addition of* cyclo*hexylamine.*

Aluminium 1-methylheptyloxide, 0·171M; octan-2-one, 0·151M.

| Amine molarity | 0 | 0·500 | 1·00 |
|---|---|---|---|
| $a_D^{30\cdot0}$ initial | −1·10° | −0·63° | −0·46° |
| $a_D^{30\cdot0}$ at *ca.* 300 min. | −0·63° | −0·65° | −0·41° |

TABLE 7. *Racemization of* (+)-*aluminium* 1-*methylheptyloxide by octan-2-one in benzene solution.*

| | Alkloxide molarity, 0·555 Ketone " 0·667 | | | | Alkoxide molarity, 0·555 Ketone " 0·800 | | |
|---|---|---|---|---|---|---|---|
| $t$ (min.) | $a^{13}$ | $t$ (min.) | $a^{13}$ | $t$ (min.) | $a^{14\cdot5}$ | $t$ (min.) | $a^{14\cdot5}$ |
| 2 | −0·20° | 30 | −1·25° | 7 | −1·14° | 210 | −1·075° |
| 7 | −0·45 | 41 | −1·23 | 8 | −1·29 | 289 | −0·925 |
| 9 | −0·88 | 132 | −1·16 | 10·5 | −1·34 | 402 | −0·79 |
| 14 | −1·17 | 339 | −0·95 | 16 | −1·36 | 800 | −0·52 |
| 20 | −1·23 | 1322 | −0·40 | 81 | −1·225 | | |

The results of certain preliminary experiments utilizing impure aluminium 1-methylheptyl-oxide—preparation " (*b*) "—are recorded in Table 7. The results, which probably arise from the impure nature of the alkoxide, have no significance as far as the present investigation is concerned, but have been included as they represent a rather unusual case of mutarotation and indicate the complications which may arise in the study of more complex systems.

## Discussion

It has been observed that, for both examples examined, the rate of racemization is dependent on the ratio of the concentrations of the alkoxide and ketone, *i.e.*, on the factor [A]/[B] (cf. equation 5). This result appears to be consistent with the postulated mechanism provided the associated nature of aluminium alkoxides is borne in mind. If it is assumed that the rate of hydrogen transfer (step 2) is slow compared with the rate at which the initial equilibrium is attained, it follows that the rate of racemization ($R$) is given by equation (9). The concentration of optically active complex is determined by the result of the

$$R = k_1[(+)\text{-Complex}] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

initial co-ordination equilibrium (step 1) which, however, is in competition with another equilibrium process, namely, the association of the aluminium alkoxide. The association reaction depends on the square or higher powers of the alkoxide concentration and may therefore be expected to compete more favourably at higher alkoxide concentrations. It is thus possible that changes in alkoxide concentration over the limited range examined have little or no effect on the concentration of the complex, the increased tendency for co-ordination being offset by the greatly increased tendency towards association. Equation (5) therefore becomes intelligible. It is possible that the observed initial inconsistencies (Figs. 2 and 3) are due to the establishment of the co-ordination and association equilibria.

The addition of a strong base to the reaction mixture introduces a third equilibrium, a process which is also competing for free alkoxide, and thus reduces the rate of racemization. Furthermore, the formation of a new optically active species, the alkoxide–amine complex, would be expected to have an effect on the initial rotation of the mixture. These conclusions are substantiated by the results recorded in Table 7. The amine greatly reduces the initial rotation, and further, this change is very rapid, indicating that the co-ordination reactions are fast. It is also seen from Table 7 that the racemization is almost completely inhibited by the addition of the amine. There are, however, several examples of successful reductions of amino-ketones (Lions *et al.*, *J. Proc. Roy. Soc. N.S. Wales*, 1939, **72**, 233, 280; Lutz *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 2020; 1949, **71**, 478), although Lutz and his co-workers (*ibid.*, 1950, **72**, 4085; 1951, **73**, 1639) found that the Oppenauer oxidations of certain amino-alcohols do not proceed at measurable speeds. It is probable that racemization in the presence of *cyclo*hexylamine would occur more rapidly at 80°, the usual temperature at which the Meerwein–Ponndorf reduction is carried out. The basic strength of the amine must also be a critical factor.

It is surprising to find such large differences in both the energies and the entropies of activation of the two processes examined. If the above interpretation of the kinetics is correct, the experimental parameters will refer to complex combinations of the several factors:

$$\Delta E_{obs.} = \alpha + \Delta E^{\ddagger} \qquad \Delta S_{obs.} = \beta + \Delta S^{\ddagger}$$

Thus $\alpha$ and $\beta$ may be interpreted as the heat and entropy of dilution of the alkoxide in a mixture of ketone and methyl*cyclo*hexane, while $\Delta E^{\ddagger}$ and $\Delta S^{\ddagger}$ refer to the hydrogen transfer through one configuration of the transition state. Experiments with $^{14}C$-labelled ketones are being undertaken to examine the path through the other configuration.

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## 623. *Studies of Coal Tar Bases. Part VI.* Hydrogenation of 2-Methylpyridine.*

By J. Idris Jones and A. S. Lindsey.

Use of W6 Raney nickel for hydrogenation of 2-methylpyridine at 150° leads to improved yields of 2-methylpiperidine. At higher temperatures hydrogenolysis of 2-methylpiperidine occurs. 1-*n*-Amylpiperidine, *n*-pentane, *cyclo*pentane, methyl*cyclo*pentane, and *n*-hexane have been identified among the products, and possible structures for other basic products are discussed. The origin of 6-methyl-2-piperidone, which is also formed, is ascribed to the absorption of atmospheric carbon dioxide by the base before the reaction and arises by hydrogenolysis of the intermediate 1-formyl-2-methylpiperidine.

In a study of the catalytic hydrogenation of 2-methylpyridine we have confirmed McElvain and Carney's report (*J. Amer. Chem. Soc.*, 1946, **68**, 2592) that at 200°/150—300 atm. over W2 Raney nickel 2-methylpiperidine is obtained in only 45% yield after careful fractionation of the product. However, by using the more active W6 Raney nickel (Adkins and Billica, *ibid.*, 1948, **70**, 695) hydrogenation proceeds readily at 140—150°/200 atm., giving 70—75% yields of pure 2-methylpiperidine, owing to the smaller amount of hydrogenolysis at the lower temperature. As with pyridine (*J.*, 1950, 1392) considerable hydrogenolysis occurs with Raney nickel at $\not<200°$ and this paper describes an examination of the hydrogenolysis products. In general, the results indicate that cleavage and condensation reactions similar to those described for pyridine occur but there is an even greater variety of products owing to the dissymmetry of the molecule and the yields of individual compounds are smaller.

Presence, in the products, of 1-*n*-amylpiperidine, previously shown to be a product of hydrogenolysis of piperidine, shows that the methyl group is fairly readily removed. *n*-Pentane and *cyclo*pentane are found, presumably resulting by elimination of ammonia from the piperidine, followed by hydrogenation and cyclisation respectively. Similarly, *n*-hexane and methyl*cyclo*pentane, also present, are derived by analogous elimination of ammonia from 2-methylpiperidine. One of the high-boiling products was 6-methyl-2-piperidone, the nature of which followed from its dehydrogenaton to 6-methyl-2-pyridone and direct comparison with an authentic specimen (Bunzel, *Ber.*, 1889, **22**, 1056).

A likely source of the combined oxygen was atmospheric carbon dioxide previously absorbed by the base, since examination of the hydrogen employed revealed only traces of carbon dioxide: indeed, hydrogenation of 2-methylpyridine in the presence of carbon dioxide gave 6-methyl-2-piperidone, in low yield. Farlow and Adkins (*J. Amer. Chem. Soc.*, 1935, **57**, 2222) have shown that in the presence of amines, carbon dioxide is readily reduced to formic acid and that at $>100°$ formylation of the amine occurs. With 2-methylpiperidine, 1-formyl-2-methylpiperidine is first formed. This then undergoes cleavage with elimination of the α-carbon atom, followed by recyclisation to 6-methyl-2-piperidone. In support of this view, 1-formyl-2-methylpiperidine on hydrogenolysis under conditions similar to the above affords 6-methyl-2-piperidone in about 5% yield. Hydrogenation of piperidine in presence of carbon dioxide gives 1-formylpiperidine but no piperidone; and hydrogenolysis of 1-formylpiperidine did not lead to detectable amounts of piperidone. It is possible that in this case, where there is no "protecting" methyl group, piperidone is hydrogenated to piperidine as soon as it is formed. Formation of 6-methyl-2-piperidone under the conditions described affords striking confirmation of the cleavage reaction observed in the previous work on piperidine (*loc. cit.*), where similar elimination of the α-carbon atom of the ring led to the formation of 1-*n*-butylpiperidine in appreciable quantity.

Four tertiary bases, $C_7H_{15}N$, $C_{10}H_{21}N$, $C_{10}H_{21}N$, and $C_{11}H_{21}N$ were also isolated in the experiments with 2-methylpiperidine, but their identities have not been definitely established. The $C_7$ base, which is neither 1-ethyl- nor 1 : 2-dimethyl-piperidine, is

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probably (? *trans*-)1 : 2 : 5-trimethylpyrrolidine, as we have found 1 : 2-dimethylpyrrolidine among the low-boiling products of pressure hydrogenolysis of piperidine; comparison of the $C_7$ base with a synthetic sample of (? *cis*-)1 : 2 : 5-trimethylpyrrolidine was, however, not conclusive. By direct comparisons, the $C_{10}$ base (picrate, m. p. 96—97°) was shown not to be identical with 1-*n*-, or 1-*sec*.-amyl- or 1-*n*- or 1-*sec*.-butyl-2-methylpiperidine. The infra-red absorption spectrum of this base showed an ethylenic bond to be present, which suggests that it may be an alkenyldialkylamine.

Some derivatives of a number of 1-alkylpiperidines, prepared by standard methods during this investigation, are described in the Experimental section. Of these, 1-*sec*.-amyl- and 2-methyl-1-*cyclo*pentyl-piperidine appear to be new.

## Experimental

Microanalyses were carried out by Miss M. Corner of this Laboratory and by Drs. Weiler and Strauss, Oxford. M. p.s are uncorrected.

*Hydrogenation of 2-Methylpyridine.*—Typical hydrogenations were as follows:

(a) *Using* W2 *Raney nickel.* 2-Methylpyridine, b. p. 129—130° (2100 g.), containing suspended W2 Raney nickel (200 g.) (*Org. Synth.*, **21**, 15), was hydrogenated in a stainless-steel autoclave at 200°/200 atm. for 24 hours. The liquid product (1886 g.) was fractionated through a Stedman column (equiv. to 50 theoretical plates), giving a main fraction of 2-methylpiperidine (1045 g., 47%), b. p. 117—120°, m. p. −4·5°, $n_D^{20}$ 1·4465.

(b) *Using* W6 *Raney nickel.* 2-Methylpyridine (1000 g.) containing suspended W6 Raney nickel (100 g.) (Adkins and Billica, *loc. cit.*) was hydrogenated at 150°/200 atm. for 10 hours with approx. theoretical absorption of hydrogen. The liquid product (996 g.) was fractionated as before, giving a main fraction of 2-methylpiperidine (780 g., 73%), b. p. 114—114·5°/700 mm., $n_D^{20}$ 1·4464.

The purity of these products was apparent from the close correspondence between their physical constants and infra-red absorption spectra with those of pure 2-methylpiperidine, b. p. 116—118°, $n_D^{20}$ 1·4460, obtained by regeneration from the repeatedly crystallised 1-benzoyl derivative (m. p. 48°).

*Pressure Hydrogenolysis of 2-Methylpiperidine.*—2-Methylpiperidine (1000 g.), b. p. 117—120°, $n_D^{20}$ 1·4465, when heated with W2 Raney nickel (100 g.) under hydrogen in a steel autoclave for 6 hours at 250°/200 atm. (total time of heating 12 hours), yielded a mixture of gaseous bases and paraffins (*ca.* 160 g.), and a strongly ammoniacal-smelling liquid (843 g.), which on distillation gave a low-boiling fraction (*A*) (b. p. <140°; 711 g.), and a high-boiling fraction (*B*) (b. p. >140°; 130 g.). These were refractionated.

*Examination of fraction* (*A*). Subfraction 1 (31 g.), b. p. 35—90°, on treatment with hydrochloric acid gave a neutral fraction (11 g.) and recovered bases (20 g.), the latter not being further examined. Distillation of the neutral fraction gave peak fractions, (i) b. p. 40—43°, $n_D^{20}$ 1·3698, aniline point 62° (2·3 g.) (Found: C, 83·9; H, 16·2%), and (ii) b. p. 63—65°, $n_D^{20}$ 1·3874, aniline point 51° (1·8 g.) (Found: C, 84·4; H, 15·45%).

The analyses, refractive indices, and aniline points indicated that fraction (i) was a mixture of *n*-pentane 80% (approx.) and *cyclo*pentane, and that fraction (ii) was a mixture of *n*-hexane 60% (approx.) and methyl*cyclo*pentane. This was confirmed by their infra-red absorption spectra.

Subfraction 2 (38·5 g.), b. p. 90—92°, was shown by titration with acid to be a water–base azeotrope containing 30—40% of base. By saturation with sodium hydroxide, the supernatant bases were separated and then dried (NaOH). The dry bases (2 g.) in 20% sodium hydroxide solution (10 ml.) were treated with toluene-*p*-sulphonyl chloride (4·5 g.). Separation by the Hinsberg method gave a trace of primary base; the toluene-*p*-sulphonate (3·8 g.), m. p. 54°, of a secondary base crystallised from alcohol, giving no depression in m. p. on admixture with authentic 2-methyl-1-toluene-*p*-sulphonylpiperidine (m. p. 55—56°), and a tertiary base (0·5 g.), which yielded a *picrate*, m. p. 183—184° (Found: C, 45·8; H, 5·2; N, 16·4. $C_7H_{15}N,C_6H_3O_7N_3$ requires C, 45·6; H, 5·3; N, 16·4%), a *hydrochloride*, m. p. 286—287° (Found: C, 56·6; H, 10·95; Cl, 23·6. $C_7H_{15}N,HCl$ requires C, 56·2; H, 10·8; Cl, 23·7%), and a *methiodide*, m. p. 318° (decomp.) (Found: C, 37·6; H, 7·3; N, 5·7. $C_7H_{15}N,CH_3I$ requires C, 37·7; H, 7·1; N, 5·5%). Admixture of the above picrate with picrates of 1-ethyl- and 1 : 2-dimethyl-piperidine, and (? *cis*-)1 : 2 : 5-trimethylpyrrolidine (see below) gave depressions of the m. p.

Subfraction 3, b. p. 116—120° (423 g.), was mainly 2-methylpiperidine (infra-red spectrum).

Subfraction 4, b. p. 120—122° (31·5 g.), was not investigated.

*Examination of fraction* (*B*). Subfraction 5, b. p. <40°/3 mm. (17 g.), was not investigated.

Subfraction 6 (9·5 g.), b. p. 40—60°/3 mm., with toluene-*p*-sulphonyl chloride gave a tertiary base (80%) which yielded a picrate, m. p. 109° (Found: C, 49·6; H, 6·2; N, 14·7. Calc. for $C_{10}H_{21}N,C_6H_3O_7N_3$: C, 50·0; H, 6·3; N, 14·7%), giving no depression in m. p. on admixture with 1-*n*-amylpiperidine picrate, m. p. 110°.

Subfraction 7 (7 g.), b. p. 60—70°/3 mm., with toluene-*p*-sulphonyl chloride gave tertiary bases (90%), which on distillation yielded a main fraction, b. p. 185°, $n_D^{20}$ 1·4506 (Found: C, 77·0; H, 13·5; N, 9·2. $C_{10}H_{21}N$ requires C, 77·3; H, 13·6; N, 9·0%), yielding a *picrate*, m. p. 96—97° (Found: C, 50·4; H, 6·3; N, 14·7. $C_{10}H_{21}N,C_6H_3O_7N_3$ requires C, 50·0; H, 6·25; N, 14·7%), a *methiodide*, m. p. 193—194° (Found: I, 41·8. $C_{10}H_{21}N,CH_3I$ requires I, 41·7%), and a very hygroscopic hydrochloride, m. p. 165—167°. Admixture of this picrate with picrates of 1-*n*- or 1-*sec.*-amyl- or 1-*n*- or 1-*sec.*-butyl-2-methyl-piperidine led to m. p. depressions.

The infra-red absorption spectrum of the free tertiary base showed a moderately intense band at 6·02 μ. This was too weak to be due to acyclic C=N and can be assigned therefore to C=C.

Subfraction 8 (11 g.), b. p. 80—82°/3 mm., with toluene-*p*-sulphonyl chloride gave tertiary bases (90%), which on distillation yielded main fractions: (i) b. p. 140—150°, $n_D^{20}$ 1·4528, giving a *methiodide*, m. p. 188—189° (Found: C, 46·6; H, 8·0; N, 4·45; I, 40·5. $C_{11}H_{21}N,CH_3I$ requires C, 46·6; H, 7·8; N, 4·5; I, 41·1%); and (ii) a *base*, b. p. 190°, $n_D^{20}$ 1·4555 (Found: C, 77·7; H, 13·7; N, 9·0. $C_{10}H_{21}N$ requires C, 77·3; H, 13·7; N, 9·0%), giving a *picrate*, m. p. 76° (Found: C, 50·1; H, 6·5; N, 14·2. $C_{10}H_{21}N,C_6H_3O_7N_3$ requires C, 50·0; H, 6·3; N, 14·7%).

Subfraction 9 (12 g.), b. p. 115—120°/3 mm., had m. p. 88—89° after recrystallisation from light petroleum (b. p. 40—60°) and repeated sublimation *in vacuo* (Found: C, 64·0, 63·2; H, 9·6, 9·8; N, 12·0, 12·6. Calc. for $C_6H_{11}ON$: C, 63·7; H, 9·8; N, 12·4%), m. p. not depressed on admixture with authentic 6-methyl-2-piperidone, m. p. 88°, prepared by Bunzel's method (see below). It formed a *picrate*, m. p. 72—73° (from ether) (Found: C, 41·9; H, 4·1; N, 15·8. $C_6H_{11}ON,C_6H_3O_7N_3$ requires C, 42·1; H, 4·1; N, 16·4%), a *hydrochloride*, m. p. 150—151° (Found: Cl, 23·6. $C_6H_{11}ON,HCl$ requires Cl, 23·75%), and a methiodide, m. p. 156—157° (decomp.). The infra-red absorption spectrum of the solid, m. p. 88—89°, was identical with that of the synthetic specimen of 6-methyl-2-piperidone. The solid (500 mg.) intimately ground with palladium black (100 mg.) was heated at 260—280° for 3 hours. The solid product was extracted with ether. Two fractional sublimations at 80°/1 mm. gave as main product 6-methyl-2-pyridone, m. p. 159—160° (Found: C, 66·1; H, 6·5; N, 12·8. Calc. for $C_6H_7ON$: C, 66·1; H, 6·5; N, 12·8%) [picrate, m. p. 146—147° (Found: C, 42·6; H, 3·0; N, 16·6. $C_6H_7ON,C_6H_3O_7N_3$ requires C, 42·6; H, 3·0; N, 16·6%); dibromo-derivative, m. p. 243—244° (Found: Br, 60·5. Calc. for $C_6H_5ONBr_2$: Br, 59·9%)]. For 6-methyl-2-pyridone Errera (*Ber.*, 1900, **33**, 2969) gives m. p. 159°, and for its dibromo-derivative m. p. 238—239°.

A still residue, consisting of a brown tar (50 g.), was not examined.

*Synthesis of* 6-*Methyl*-2-*piperidone* (cf. Bunzel, *loc. cit.*).—Potassium permanganate oxidised 1-benzoyl-2-methylpiperidine to 5-benzamidohexanoic acid, m. p. 151—152° (Found: C, 66·3; H, 7·4; N, 5·9. Calc. for $C_{13}H_{17}O_3N$: C, 66·4; H, 7·3; N, 6·0%), which on dry distillation at 268—270°, followed by treatment with aqueous sodium hydroxide and extraction with chloroform, gave 6-methyl-2-piperidone, m. p. 88°, after sublimation at 80°/1 mm. (Found: C 63·4; H, 9·5; N, 12·5. Calc. for $C_6H_{11}ON$: C, 63·7; H, 9·8; N, 12·4%).

*Preparation of* 6-*Methyl*-2-*piperidone by Hydrogenation of* 2-*Methylpyridine and Carbon Dioxide*.—Hydrogenation of 2-methylpyridine (200 g.) and carbon dioxide (20 g.) over Raney nickel (20 g.) at 180°/150 atm. for 11 hours and distillation of the products gave crude 6-methyl-2-piperidone (4 g.). Repeated crystallisation from light petroleum (b. p. 40—60°) and sublimation gave a product, m. p. 86—87°. The infra-red absorption spectrum was identical with that of the specimen made by Bunzel's synthesis.

*Preparation of* 6-*Methyl*-2-*piperidone by Hydrogenolysis of* 1-*Formyl*-2-*methylpiperidine*.—1-Formyl-2-methylpiperidine, b. p. 227—228° (101 g.) [mercurichloride, m. p. 100—101° (Found: N 3·6. Calc. for $C_7H_{13}ON,HgCl_2$: N, 3·5%)], was hydrogenolysed over Raney nickel (10 g.) for 7 hours at 250°/160 atm. Fractional distillation gave a crude solid (6 g.), b. p. 90—110°/0·5 mm., which after recrystallisation and sublimation had m. p. 88—89°, alone or on admixture with authentic 6-methyl-2-piperidone, and possessed an infra-red absorption spectrum identical with that of the authentic compound.

*Hydrogenolysis of Piperidine in the Presence of Carbon Dioxide.*—Piperidine (100 g.) containing suspended Raney nickel (10 g.) was placed in a 1-l. steel autoclave, which was then filled with carbon dioxide at 50 atm., followed by hydrogen to a pressure of 160 atm. After 4 hours' heating at 150°/250 atm., the mixture of solid carbonates obtained was dissolved in the minimum quantity of water, filtered, and acidified with concentrated hydrochloric acid. After basification of the mixture with an excess of solid sodium hydroxide ether-extraction gave a yellow oil (110 g.), which on distillation gave mainly unchanged piperidine (62 g.) and 1-formylpiperidine (17 g.), b. p. 215—218° (mercurichloride, m. p. 148—149° alone or on admixture with an authentic sample).

*Preparation of* 1-*Alkylpiperidines* [with S. E. Hunt].—Various 1-alkylpiperidines were prepared from the free base and the alkyl bromide by Magnusson and Schierz's method (Univ. of Wyoming Publ., 1940, vol. 7, p. 1). 1-*sec.*-Amylpiperidine, b. p. 190°, $n_D^{20}$ 1·4505 [*picrate*, m. p. 93—94° (Found: C, 50·1; H, 6·2; N, 14·4. $C_{10}H_{21}N,C_6H_3O_7N_3$ requires C, 50·0; H, 6·25; N, 14·7%)], was prepared by Leuckart alkylation of piperidine with methyl *n*-propyl ketone (cf. Smith and Macdonald, *J. Amer. Chem. Soc.*, 1950, **72**, 1037). 1-*cyclo*Pentyl-2-methylpiperidine, b. p. 220—224°, $n_D^{20}$ 1·4834 [*picrate*, m. p. 129—130° (Found: C, 51·5; H, 6·1; N, 13·9. $C_{11}H_{21}N,C_6H_3O_7N_3$ requires C, 51·5; H, 6·1; N, 14·1%); methiodide, m. p. 193—194°] was prepared by heating *cyclo*pentanone and 2-methylpiperidine with Raney nickel and hydrogen (100 atm.) at 140° for 6½ hours. The other compounds prepared were: 1 : 2-dimethyl-, b. p. 129°, $n_D^{20}$ 1·4412 (picrate, m. p. 246°), 2-methyl-1-*n*-propyl-, b. p. 168—170°, $n_D^{20}$ 1·4474 (picrate, m. p. 118—119°), 1-*sec.*-butyl-2-methyl-, b. p. 174° (picrate, m. p. 139°), 1-*n*-butyl-2-methyl-, b. p. 187°, $n_D^{20}$ 1·4509 (picrate, m. p. 114—115°; methiodide, m. p. 218—219°), and 1-*n*-amyl-2-methyl-piperidine, b. p. 204—206°, $n_D^{20}$ 1·4528 (picrate, m. p. 94—95°; methiodide, m. p. 197—198°).

*Preparation of* (? cis-)1 : 2 : 5-*Trimethylpyrrolidine.*—2 : 5-Dimethylpyrrole (*Org. Synth.*, **16**, 25) was hydrogenated over Adams's catalyst in ethanol–acetic acid (1 : 1). The recovered base on distillation gave 2 : 5-dimethylpyrrolidine, b. p. 105°, converted into 1 : 2 : 5-trimethylpyrrolidine by methyl iodide. The free base yielded a *picrate*, m. p. 209—210° (Found: C, 45·85; H, 5·2; N, 16·6. $C_7H_{15}N,C_6H_3O_7N_3$ requires C, 45·6; H, 5·3; N, 16·4%).

The work described above formed part of the programme of the Chemical Research Laboratory and is published by permission of the Director. We thank Mr. J. L. Hales for performing the infra-red absorption analyses, and Messrs. E. C. Holt and A. Sleven for carrying out much of the distillation work.

Chemical Research Laboratory, D.S.I.R., Teddington. [*Received, March 31st*, 1952.]

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## 624. *The Hydrolysis of Amides of Dibasic Acids. Part I. The Acid Hydrolysis of Malonamide and Malonamic Acid.*

By H. G. Ivory, W. D. McKenzie, J. Packer, and J. Vaughan.

Rates of hydrolysis, in excess of hydrochloric acid (HCl = 0·0625—0·75M), of malonamide and malonamic acid have been measured at 80° and 98°. The rate of hydrolysis of malonamide, per amide group, is approximately the same at each stage of the process. Results with malonamic acid appear incompatible with Hall's conclusions (*J. Amer. Chem. Soc.*, 1950, **72**, 4709) on the behaviour of this compound in aqueous acid at 80—90°.

The course of the acid hydrolysis of malonamide would be expected to produce malonamic acid as an intermediate:

$$CH_2(CO{\cdot}NH_2)_2 \xrightarrow[k_1]{\text{Step I}} CH_2(CO{\cdot}NH_2){\cdot}CO_2H \xrightarrow[k_2]{\text{Step II}} CH_2(CO_2H)_2$$

In sufficient excess of acid, kinetics should be of first-order and should be consistent with the existence of two consecutive reactions, provided that the rates of these reactions are not too different. Although theoretical considerations and existing data affirm that the electron-attracting power of the carboxyl group is superior to that of the amide group under comparable conditions, the difference in influence of these groups on the rates of

hydrolysis of a neighbouring amide group (*e.g.*, in malonamide and malonamic acid) should not be excessive. Hence, the reaction rates $k_1$ and $k_2$ should be comparable.

Swain (*J. Amer. Chem. Soc.*, 1944, **66**, 1696) has analysed mathematically the kinetics of consecutive first-order reactions and has concluded that the initial slope of the " ln (concn. of reactant)–time " graph is $\frac{1}{2}k_1$ and that the final slope is $k_2$. When $k_1 = 2k_2$, therefore, the graph should be linear throughout. When such a linear plot is obtained, Swain's relationships may be demonstrated by a simple treatment. If $a$ represents the initial molar concentration of unhydrolysed amide, $\frac{1}{2}a$ is the sum quantity of amide available for Step I. If the fraction (of total amide) which has reacted at time $t$ is given by $x$, then at the beginning of reaction, when $t$ and $x$ are small,

$$-k_1 = \mathrm{d}\ln(\tfrac{1}{2}a - x)/\mathrm{d}t$$
$$= \mathrm{d}\ln(1 - 2x/a)/\mathrm{d}t + \mathrm{d}(\ln \tfrac{1}{2}a)/\mathrm{d}t = \mathrm{d}(-2x/a)/\mathrm{d}t$$

[since the general function $\ln(1 - m) = -m$ when $m$ is small].

If $k$ applies to the overall reaction rate as determined experimentally,

$$-k = \mathrm{d}\ln(a - x)/\mathrm{d}t = \mathrm{d}(-x/a)/\mathrm{d}t$$

whence $k \longrightarrow \frac{1}{2}k_1$ as $t \longrightarrow 0$. As $t \longrightarrow \infty$, $a$ and $x$ are almost equal and Step I is almost complete. Then for Step II :

$$-k = \mathrm{d}\ln[\tfrac{1}{2}a - (x - \tfrac{1}{2}a)]/\mathrm{d}t$$

But as $t \longrightarrow \infty$, $(x - \frac{1}{2}a) \longrightarrow \frac{1}{2}x$ and therefore

$$-k_2 = \mathrm{d}\ln(\tfrac{1}{2}a - \tfrac{1}{2}x)/\mathrm{d}t = \mathrm{d}\ln(a - x)/\mathrm{d}t$$

whence $k \longrightarrow k_2$ as $t \longrightarrow \infty$.

The fact that $k_1 \longrightarrow 2k$ as $t \longrightarrow 0$ is important. The presence of two amide groups doubles the probability of reaction occurring; $k$ thus measures the rate of hydrolysis per amide group, and the whole plot of $\ln(a - x)$ against time shows the average rate of hydrolysis *per amide group* at any time during the reaction. In many cases where $k_1 \neq 2k_2$ the $\ln(a - x)$–time graph will not give $k_1$ and $k_2$ very accurately. Swain (*loc. cit.*) has developed a graphical technique by means of which $k_1$ and $k_2$ may be measured to within an accuracy of 1%. In some instances, however, the original graph gives $k_1$ with reasonable accuracy, and $k_2$ may be obtained by separate hydrolysis of the intermediate compound.

In the work here reported, a study of the kinetics of hydrolysis, in excess of hydrochloric acid at temperatures of 80° and 98°, of both malonamide and malonamic acid has been carried out, and the results are discussed in relation to some rates of decarboxylation of malonamic acid measured by Hall (*loc. cit.*).

## Experimental

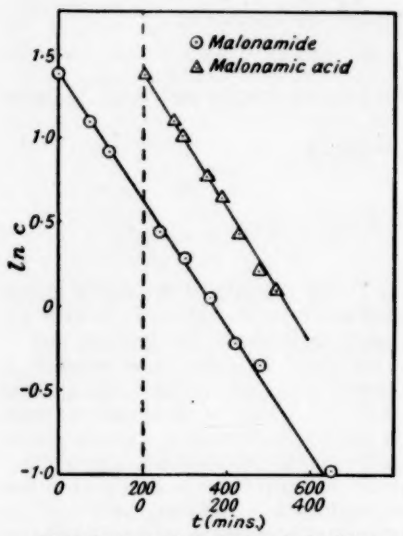
In general, the products of amide hydrolysis are the parent acid and ammonia, and estimation of the ammonia formed is a convenient means of following reaction rates. Earlier work in these laboratories has confirmed that in reactions of this type the ammonia may be measured accurately by hypobromite methods based on work by Taylor (*J.*, 1930, 2741) and by Krieble and Holst (*J. Amer. Chem. Soc.*, 1938, **60**, 2976). Two slightly different techniques need to be used for different amides.

(*a*) When no interaction is detected between unhydrolysed amide and alkaline hypobromite a direct titration method can be followed. In the present work the following procedure was adopted. A sample of the solution was withdrawn from the reaction flask and cooled to below 20° to prevent appreciable further hydrolysis. A 20-ml. aliquot was partly neutralised with a 10% solution of sodium hydroxide and neutralisation completed with a 1% solution, methyl-red being used as indicator. 10 Ml. (excess) of standard hypobromite solution were added and allowed to react for 3 minutes before the addition of 2 ml. of 5% potassium iodide solution and 5 ml. of 6N-sulphuric acid. The liberated iodine was titrated with 0·01N-sodium thiosulphate, with starch as indicator.

(*b*) If it is found that reaction occurs between the amide and hypobromite, the ammonia must be distilled off for analysis. We used a modification of Pucher, Vickery, and

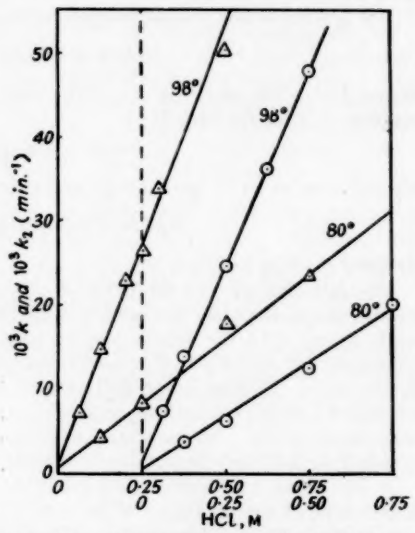
Leavenworth's method (*Ind. Eng. Chem. Anal.*, 1935, **7**, 152), the steam-distillation flask being equipped with a long neck, and extra precautions being taken to minimise splashing. 20 Ml. of the cold hydrolysate were placed in the distillation flask and neutralised as in method (*a*). 25 Ml. of 0·02N-sulphuric acid were run into the receiver, and the apparatus was assembled. The distilling flask was kept at 40°, and the receiver cooled in ice. Immediately before distillation was started, 4 ml. of the borax–sodium hydroxide buffer solution were added, and distillation was carried out at 20 mm. and for 15 minutes. A slow current of dry air was drawn through a wash-bottle containing 0·1N-sulphuric acid, and then through the apparatus, for the whole of this period. The distillate was titrated as in method (*a*), the titration being rapidly carried out, after acidification of the iodide solution, to avoid any possible " after-blueing " near the end-point. The hypobromite solution was, in each method, standardised beforehand by a complete reproduction of the processes involved, a 20-ml. aliquot of the hydrochloric acid, of appropriate strength, being used in place of the hydrolysate. The sensitivity of the analysis demands

FIG. 1.




*Hydrolysis at 80° in 0·125M-hydrochloric acid.*

FIG. 2.




*Dependence of rate constant on molarity of acid : ⊙ = malonamide, △ = malonamic acid.*

scrupulous care and practice to give reproducible and accurate results when dealing with quantities of ammonia below 1 mg. Standard ammonium sulphate solution has been used as a gauge of reliability and due attention to details has resulted in completely satisfactory analyses, the error being ±2% over a range of 0·1—0·5 mg. of ammonia.

Hydrolyses were carried out in an all-glass Pyrex apparatus, at either 80° ± 0·5° or 98° ± 0·5°, the requisite amount of amide being introduced into the acid solution after the latter had reached temperature equilibrium. Dissolution time was extremely short as compared with total reaction time, and its effect on the kinetics was negligible. First-order kinetics were assured by the use of a concentration ratio of mineral acid to amide of $\geqslant$100 : 1, and an amide concentration giving 0·2 mg. of nitrogen per aliquot of hydrolysate proved convenient.

Malonamide, recrystallised three times from 80% aqueous alcohol, had m. p. 170°. There was no detectable reaction with hypobromite, and the direct titration method was adopted. In each hydrolysis run the plot of $\ln(a - x)$ against time was linear for the whole of the hydrolysis (Fig. 1) and this fact indicated that, under our experimental conditions, any difference in the rates of hydrolysis of the two amide groups was not detectable. The pseudo-unimolecular nature of the hydrolysis was confirmed by varying the ratio of amide to acid without alteration in the $k$ values obtained. Hydrolyses were followed within the range HCl = 0·0625—0·75M.

Repetitive runs carried out at each acid concentration showed reproducibility to be satisfactory. The mean of the rate constants obtained at a given acid concentration was used to define the corresponding point on a plot of $k$ against HCl concentration, and the plots obtained were linear: they (Fig. 2 and Table) could be defined by the equations:

$$(80°)\ k = 0{\cdot}0002 + 0{\cdot}026[\mathrm{HCl}]$$
$$(98°)\ k = 0{\cdot}002 + 0{\cdot}093[\mathrm{HCl}]$$

The approximate energy of activation is 18·4 kcal. mole$^{-1}$.

Malonamic acid was prepared by Jeffery and Vogel's method (*J.*, 1934, 1102) and after recrystallisation had m. p. 118°. The direct titration method could be used for the hydrolyses, and the experimental range was the same as that covered with malonamide. The kinetics were

| | Rate constant × $10^3$ (min.$^{-1}$) | | | |
|---|---|---|---|---|
| | Malonamide, $k$ | | Malonamic acid, $k_2$ | |
| [HCl], M | 80° | 98° | 80° | 98° |
| 0·0625 | — | 7·0, 7·5, 7·2, 7·8 | — | 7·1, 7·2 |
| 0·125 | 3·85, 3·67, 3·85 | 13·4, 13·8, 13·7 | 4·08, 4·08 | 14·8, 15·1, 14·4 |
| | 3·81, 3·73, 3·50 | 14·4, 13·8, 14·4 | | 14·5 |
| | 3·73, 3·46, 3·46 | 13·8, 14·4 | | |
| 0·200 | — | — | — | 23·0, 23·0 |
| 0·250 | 6·73, 6·33, 6·42 | 23·0, 25·1, 23·5 | 8·42, 8·41 | 26·2, 26·9 |
| | 5·77, 5·93, 6·00 | 27·3 | | |
| 0·350 | — | — | — | 33·5, 34·3 |
| 0·375 | — | 35·3, 37·5 | — | — |
| 0·500 | 13·4, 11·8, 13·6 | 47·7, 48·3 | 17·0, 18·8, 17·5 | 49·6, 50·4, 50·5 |
| | 12·6, 11·7 | | | 51·1 |
| 0·750 | 20·2, 20·2 | 66·8, 68·3 | 23·8, 23·4 | — |

again consistent with a pseudo-unimolecular reaction. The plot of [HCl] against rate constant ($k_2$) was linear (Fig. 2 and Table) and could be described by the equations:

$$(80°)\ k_2 = 0{\cdot}0006 + 0{\cdot}032[\mathrm{HCl}]$$
$$(98°)\ k_2 = 0{\cdot}001 + 0{\cdot}10[\mathrm{HCl}]$$

The energy of activation is 16·5 kcal. mole$^{-1}$.

In view of Hall's findings (to be discussed later), the kinetics of hydrolysis of malonamic acid, at 80° and in 0·25M-hydrochloric acid, were also followed by the distillation method, in which distilled ammonia could be the only material being estimated. Blank experiments showed that malonamic acid itself gave no titratable distillate in the standard procedure. First-order kinetics were obtained, and the value of $k_2$ obtained by this method was $8{\cdot}42 \times 10^{-3}$ min.$^{-1}$, confirming the results of direct titration.

*Discussion.*—The kinetics results obtained are consistent with a two-stage process in which $k_1 \approx 2k_2$, $k_2$ being slightly greater than $\frac{1}{2}k_1$ in the present case. There is also a small difference in the activation energies found. Clearly, however, with the amides of malonic acid under our experimental conditions, the rate of hydrolysis of one amide group is not markedly affected by a change, from amide to carboxyl, in the other functional group.

The results obtained for the monoamide become of particular interest in connection with Hall's findings (*loc. cit.*) on the kinetics of decomposition of malonamic acid in acid solution at 80—90°, *viz.*, that even in 0·2M-hydrochloric acid solution there was no evidence of hydrolysis of the amide group, as shown by negative results in qualitative tests for ammonium ion in the reacting solution. He concluded that the reaction involved was the decarboxylation: $CO_2H{\cdot}CH_2{\cdot}CO{\cdot}NH_2 \longrightarrow CH_3{\cdot}CO{\cdot}NH_2 + CO_2$. We are unable to accept this conclusion. Apart from the fact that positive " Nessler " results were obtained on the reacting mixture, hydrolysis could actually be followed *via* the released ammonia, both by direct titration and also after a distillation under conditions which allow of negligible hydrolysis of the amide group. Reaction rates are directly proportional to molar concentration of acid and, where comparison is possible, are substantially higher than those assigned

by Hall to the decarboxylation of malonamic acid. Furthermore, unpublished preliminary experiments on the rates of similar hydrolysis of acetamide show that this amide, assumed by Hall to be one of his reaction products, is hydrolysed, at 100°, at rates much above those of malonamic acid. These findings imply that the conclusion drawn by Hall from his kinetics results is untenable.

Further work in progress includes hydrolysis studies on *C*-substituted malonamides. Mechanisms of amide hydrolysis will be discussed later in the light of these experiments, and further implications of the kinetic results given in this paper will then be included.

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## 625. *The Action of Ionizing Radiations and of Radiomimetic Substances on Deoxyribonucleic Acid. Part VI.* Physicochemical Measurements of the Action of Bischloroethylmethylamine.*

By J. A. V. BUTLER, L. GILBERT, and D. W. F. JAMES.

The mobilities and sedimentation and diffusion constants of deoxyribonucleic acid, which has been treated with bischloroethylmethylamine, have been determined after various periods of reaction. The molecular size decreases for a considerable time after the initial reaction. The initial change of viscosity appears to be independent of this, at least at pH 7, and is ascribed to changes of interaction and configuration consequent on the breakage of hydrogen bonds owing to the alkylation of the primary amino-groups of the bases.

IT has been shown previously (Butler and Smith, *J.*, 1950, 3411) that bischloroethylmethylamine and similar substances destroy the characteristic viscosity of deoxyribonucleic acid solutions and the change has been shown (Conway, Gilbert, and Butler, *J.*, 1950, 3421) to be accompanied by a large decrease in the apparent molecular weight deduced from sedimentation and diffusion measurements. The interpretation of these observations has been difficult because of lack of knowledge of the molecular state of the nucleic acid in these solutions, which are anomalous in many respects, in consequence of which it has not been known if the sedimenting and diffusing unit is a single molecule or an association of several and the size and shape have been in doubt. There is undoubtedly marked molecular interaction at the concentrations (*ca.* 0·05%) at which investigations of this kind are possible and it is difficult to distinguish if the effects are due to (1) a decrease of interaction between the elementary particles, (2) changes in the shape of the particles, similar to those which have been shown to occur with flexible polyelectrolytes on neutralization and addition of salts (*e.g.*, by Fuoss and Maclay, *J. Polymer Sci.*, 1951, **6**, 305; Jordan, *Trans. Faraday Soc.*, 1950, **46**, 792), or (3) a real decrease in particle size.

When the nucleic acid is treated with the amine some reaction with the phosphate "backbone" probably occurs, with the consequent partial neutralization of the nucleic acid anion, which might produce effects on the viscosity similar to those found when the ionized groups of flexible polyelectrolyte chains are neutralized. In order to find if such effects are adequate to explain the loss of viscosity of nucleic acid we determined its mobilities and sedimentation constants in the original state and after treatment with the amine, and we have also observed the change of these properties during the reaction. Although the absolute determination of charge is difficult, it was expected that it would be possible to infer from the mobilities whether there was a diminution of charge sufficient to produce the observed decrease of viscosity.

* Part V, *J.*, 1952, 834. This paper, and Part IV (*J.*, 1952, 626), were read in abstract at the XIIth International Congress of Pure and Applied Chemistry, New York, Sept., 1951.

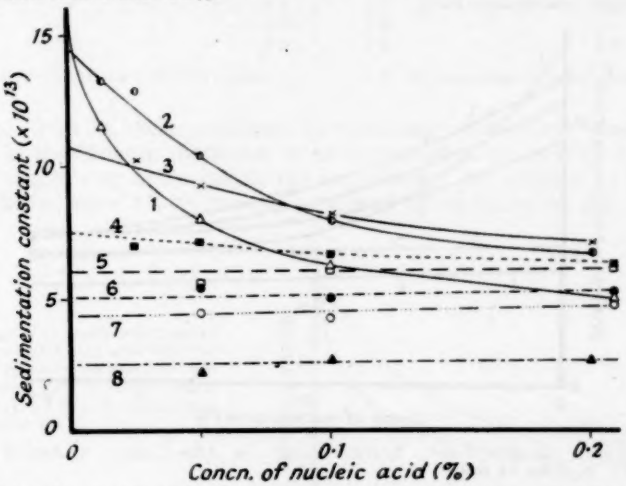
The mobilities observed with nucleic acid (G 2) and the same material treated with the amine (G 2/1) in a variety of buffer solutions are shown in Table 1.

TABLE 1. *Mobilities of nucleic acid and nucleic acid after treatment with the amine (ascending boundaries)* (20°) ($\times 10^5$ *cm.*$^2$/*sec.* V).

| pH | 4·5 | 5 | 6 | 7 | 8 | 8·5 |
|---|---|---|---|---|---|---|
| Nucleic acid (G 2) | 16·2 | 16·2 | — | 16·1 | 16·8 | 20·4 |
| Nucleic acid (G 2) treated with amine | 10·6 | 11·45 | 12·0 | 12·3 | 16·0 | — |

The proportion of the phosphate groups of the nucleic acid which have reacted with the amine and therefore become incapable of being ionized is unknown. The total amount of the amine which has reacted in our experiments was, however, known to be about 1 residue per 4 atoms of phosphorus, and since it has been shown that a part of this is combined with the bases of the nucleic acid (Press and Butler, *J.*, 1952, 626) the amount combined with the

FIG. 1. *Sedimentation constants as function of nucleic acid concentration after treatment with the amine for various times in sodium hydrogen carbonate. Reaction carried out with nucleate concentration* 0·2% *and amine concentration* 0·2%.




1, *Original nucleic acid.* 2, *After* 1 *day.* 3, *After* 3 *days.* 4, *After* 5 *days.* 5, *After* 7 *days.* 6, *After* 7 *days* + 7 *days' dialysis at* 0°. 7, *After* 18 *days, no dialysis.* 8, *After* 7 *days* + 7 *days' dialysis and freeze-drying.*

phosphate groups is unlikely to exceed half a residue of amine per tetranucleotide. The maximum possible degree of neutralization of the charged phosphate groups is thus not likely to exceed 25%.

It can be seen that the mobility of the product (G 2/1) is somewhat less than that of the parent nucleic acid at all pH's below 8.

Quantitative interpretation of these results is complicated by the possible variation of the frictional resistance to motion of the particles in the two cases. This can be eliminated by introducing the sedimentation constants in the two solutions, which have been measured in identical solutions at pH 7 only. Combining the equations $M(1 - V\rho) = fs$, and $\mu_e = ne/f$, where $M$ is the mass of the sedimenting particle, $V$ its specific volume, $\rho$ the density of the solvent, $f$ the frictional resistance, $s$ the sedimentation constant, $\mu_e$ the electrophoretic mobility, and $n$ the effective number of unit charges $e$ on the particle, we obtain

$$ne/M = (1 - V\rho)\mu_e/s$$

from which the ratio of the *effective* charge to mass ($ne/M$) of the particle can be determined.

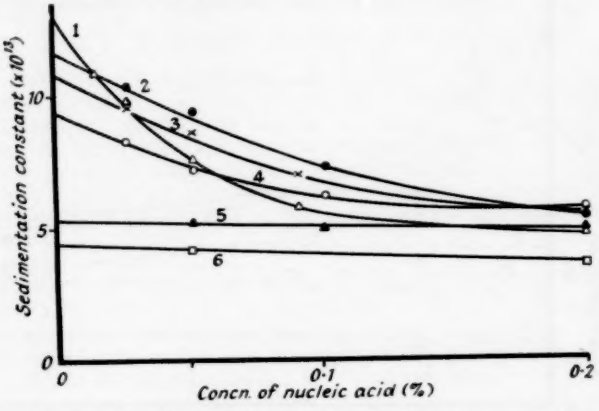
This calculation for the two products at 0·2% in 0·1M-phosphate buffer (pH 7) is given below:

| | $10^{13}s$ | $10^{5}\mu_o$ | $10^{10}ne/M$ |
|---|---|---|---|
| Nucleic acid (G 2) | 5·88 | 16·2 | 3·72 |
| Nucleic acid (treated with amine and freeze-dried) | 2·31 | 12·4 | 7·25 |

It is evident that the " effective charge " on unit mass of the material, at this pH, so far from being diminished by the reaction, is increased. The " effective charge," however, includes the charges of those counter ions which are carried along by the particles in their electrophoretic migration. The relation between the effective charge and the actual or total possible charge of particles is, in general, not known. It can only be inferred that the existence of a net effective charge implies the presence in the molecules, unless specific adsorption of ions of opposite sign is present, of more ionizable groups of the same sign.

In order to find to what extent the loss of viscosity is due to the reduction of molecular

FIG. 2. *Sedimentation constants as function of nucleic acid concentration after treatment with amine for various periods in phosphate buffer* (M/10) *at* pH 7. *Reaction carried out with nucleate concentration* 0·2% *and amine concentration* 0·2%.




1, *Control nucleic acid.* 2, *After* 1 *day.* 3, *After* 4 *days.* 4, *After* 7 *days.* 5, *After* 7 *days* + 7 *days' dialysis at* 0°. 6, *After* 14 *days.*

weight of the nucleic acid in the reaction, we determined the sedimentation constants at different times after the addition of the amine to nucleic acid in buffer solutions.

The results of these determinations, made in each case at three concentrations of nucleic acid, for the reaction in 0·1N-sodium hydrogen carbonate, are shown in Fig. 1. It can be seen that the initial effect is the disappearance of the marked concentration dependence of the sedimentation constant, combined with a decrease in the limiting value at zero concentration. This is followed by a slow downward drift of the sedimentation constants which continues for a considerable time. Since it was thought that this slow change might be due to a slow hydrolysis of the phosphate–sugar links by sodium hydrogen carbonate (pH *ca.* 8·8), similar observations were made in a phosphate buffer of pH 7 (Fig. 2). A similar sequence of events occurs, but rather more slowly, and the final sedimentation constants observed are appreciably higher than those reached in sodium hydrogen carbonate in the same time.

These decreases of sedimentation constant could be due either to decreases in molecular size or to changes of shape resulting in an *increased* resistance to motion. The fact that final molecular weights observed (from sedimentation and diffusion; *J.*, 1950, 3421) in preparations which had remained in solution for 2 weeks after the addition of amine are much lower than those of the original nucleic acid suggests that a real decrease of molecular

TABLE 2. *Diffusion and sedimentation constants of deoxyribonucleic acid during reaction with the amine.*

| Treatment | Concn. (%) | Diffusion const. ($\times 10^7$) $D_B$ | $D_M$ | Sedimentation const. ($\times 10^{13}$) | Mol. wt. |
|---|---|---|---|---|---|
| $G_3$ treated with amine (0·4%) in 0·1N-sodium hydrogen carbonate (2 days) ... | 0·2 | 1·55 | 1·60 | 7·1 | |
| | 0·1 | 1·74 | 1·41 | 8·2 | |
| | 0·05 | 1·34 | 1·50 | 9·3 | |
| | 0·0 | 1·54 * | 1·50 * | 10·8 | $4 \times 10^5$ |
| $G_3$ treated with amine (0·4%) in 0·1M-phosphate buffer at pH (2 days) ..... | 0·2 | 1·4 | 1·4 | 5·4 | |
| | 0·1 | 0·91 | 1·0 | 7·1 | |
| | 0·05 | 0·73 | 0·77 | 9·0 | |
| | 0·0 | 0·55 † | 0·66 † | 11·5 | $1 \times 10^6$ |
| $G_1$ treated with amine in sodium hydrogen carbonate for 14 days and freeze-dried (G 2/1) ..... | 0·2 | 4·5 | — | 2·5 | |
| | 0·1 | 6·4 | — | 2·5 | |
| | 0·05 | 7·0 | — | 2·5 | |
| | 0·0 | 8·0 | — | 2·5 | $1·7 \times 10^4$ |
| S similarly treated (S 1/1) ..... | 0·2 | 4·6 | — | 2·0 | |
| | 0·1 | 5·2 | — | 2·4 | |
| | 0·05 | 5·9 | — | — | |
| | 0·0 | 7·5 | — | 3·0 | $2·2 \times 10^4$ |

* Mean of figures at the different concns. † Extrapolated to zero concn.

weight occurs. This has been confirmed by some new observations given in Table 2. Measurement of the diffusion coefficients at an early stage of the reaction is difficult since appreciable changes may occur during the experiment. An attempt to determine the diffusion coefficient after 2 days' reaction, followed by equilibration with the buffer by

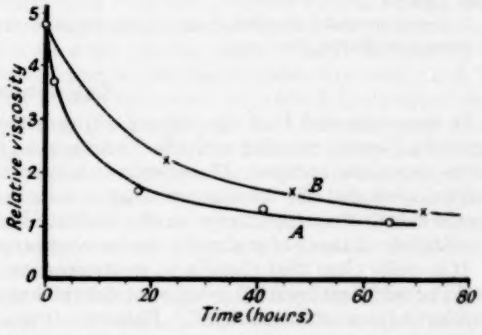



FIG. 3. *Change of viscosity with time of 0·4% thymonucleic acid with 0·4% of the amine in (A) 0·1N-sodium hydrogen carbonate and (B) 0·1M-phosphate buffer* (pH 7). *Viscosity determined after dilution to 0·1%.*

dialysis for 1 day, has given the results shown in Table 2. In view of the time taken by the diffusion experiments it has seemed desirable to use the sedimentation constant after 3 days with the diffusion constant so determined. The difference between the sedimentation constants after 2 and 3 days is, however, not large. No great reliance can be placed on the numerical values owing to the difficulties involved but they clearly indicate that while the diffusion constants are somewhat increased in the earlier stages of the reaction in the bicarbonate solution, in 2 days the sedimentation and diffusion constants in phosphate are little changed, although the viscosity has diminished considerably. Fig. 3 shows the changes of viscosity in the two cases. It follows from this that very little change of molecular weight occurs during the initial decrease of viscosity in the phosphate buffer, but that a significant decrease of molecular weight occurs in the same time in the bicarbonate buffer. The rate of change of viscosity is, however, greater in the sodium hydrogen carbonate solution and it is possible that the difference between the two cases would not be very great if the determinations were made at the same viscosity rather than at equal times.

## Experimental

*Sedimentation and Diffusion Measurements.*—The sedimentation rates were determined in the Spinco ultra-centrifuge at *ca.* 60,000 r.p.m. They are corrected to 20°. The diffusion measurements were made by examining the Schlieren diagram of the boundary between the solution and solvent in the Perkin–Elmer diffusion apparatus at various periods after the making of the boundary. The boundary was initially made by sliding the upper part of the electrophoresis cell into position and then removing the boundary into an observable position by slow displacement. Sharp initial boundaries were obtained in this way. Calculations of $D$ from the height ($D_H$) and from the breadth ($D_M$) of the Schlieren diagram at its inflection point were in good agreement (for details, see Alexander and Johnson, "Colloid Science," Oxford Univ. Press, 1949, Chap. X). The viscosity measurements were as described by Butler and Smith (*loc. cit.*).

*Mobilities.*—The mobilities were determined in the electrophoresis apparatus after careful equilibration of the solutions by dialysis with the buffers in which they were to be measured. The conductivites and pH's of the dialysed solutions and the buffers were determined to ascertain if complete equilibration had been achieved. The buffer solutions were made up to 0·1 ionic strength, as given by Miller and Golder (*Arch. Biochem.*, 1950, **29**, 420). Since the mobility might diminish in the early stages of the reaction and increase to about its initial value in the subsequent stages, an attempt was made to determine the mobility at intervals during the reaction at pH 7·4, where the final mobility is nearly the same as the initial. In order to do this, samples of the reaction mixture, taken at intervals, were dialysed against the buffer used (0·0625M-disodium hydrogen phosphate, 0·02M-sodium dihydrogen phosphate) for 3 hours only with constant agitation. The results given below (0·2% of the nucleic acid S/1) showed no significant change during the reaction:

| Time from start of reaction (hr.) | 0·0 | 9·5 | 29 | 96 |
|---|---|---|---|---|
| Mobility ($\times 10^5$) | 14·4 | 14·3 | 14·4 | 14·8 |

The nucleic acid (S) used here has a rather smaller mobility at the given pH than that referred to in Table 1.

*"Amine-treated" Nucleic Acid.*—The preparation of the samples G 2/1 and S 1/1 is described by Press and Butler (*loc. cit.*).

## Discussion

It was expected that the effective (negative) charge of the nucleate ion would be diminished by the reaction with the "nitrogen mustard," owing to the partial esterification of the phosphate groups. The effective charge was found to be increased, so that it must be concluded that the numbers of counter ions carried by the particles is diminished to a greater extent than the charge on the nucleate ions themselves. This may well happen if considerable changes of molecular size or configuration occur.

It is quite clear that there is no neutralization of the charge of the nucleate ions which would be sufficient by itself to account for the loss of viscosity, such as occurs when flexible polyelectrolytes are neutralized. However, it has been shown that 25% neutralization of the latter causes very little change of viscosity (Arnold and Overbeck, *Rec. Trav. chim.*, 1950, **69**, 192; Oth and Doty, *J. Phys. Chem.*, 1952, **56**, 43). Thus the loss of viscosity cannot be a simple consequence of the partial neutralization of the nucleate ions.

We must enquire how far it is due to the observed decreases of molecular weight. In sodium hydrogen carbonate solutions, some decrease of molecular weight occurs while the viscosity is decreasing, but in the phosphate buffer it appears that molecular weight changes very little during this period. It therefore does not seem possible to account for the loss of viscosity, at least in the phosphate buffer, as due to the breakage of the elongated molecules of nucleic acid.

The characteristic high viscosity of nucleic acid solutions is due mainly to the interaction of the nucleic acid particles with each other, giving rise even at small concentrations (>0·01%) to something resembling a gel-like net-work (Pouyet, *J. Chim. phys.*, 1951, **48**, 616; Benoit, *ibid.*, p. 612). The decrease of viscosity brought about by salts at small concentrations can be attributed mainly to the decrease of such interactions, as is shown by the change in the nature of the diffusion patterns brought about by salts (Butler and James, *Nature*, 1951, **167**, 844). It is not known with certainty how the interaction

between the particles occurs, but the fact that it is greatly reduced by even small salt concentrations would indicate that it is mainly electrostatic in character. Since reaction with the amine does not modify the electrical character of the particles very much, it is difficult to account for a marked change of viscosity in this way, although such effects may contribute.

However, the interaction of the particles may be modified as the result of the reaction for another reason. It is well known that the viscosity of nucleic acid solutions is diminished, to some extent irreversibly, by agents such as heat, acids, and alkalis (Creeth, Gulland, and Jordan, *J.*, 1947, 1141; Zamenhof and Chargaff, *J. Biol. Chem.*, 1950, **186**, 207; Chargaff, *J. Cell. Comp. Physiol.*, 1951, **38**, 41); and simple denaturing agents, such as urea and phenol, also cause irreversible changes in the nucleic acid (Conway and Butler, *J.*, 1952, 3075). Such irreversible changes have been ascribed to the breakage of intramolecular hydrogen bonds between hydroxyl and primary amino-groups of the bases in the nucleic acid particle, and it has been suggested that nucleic acid is a highly organised structure maintained by such bonds. At least, treatment with these substances gives rise to material with lower viscosities and a more compact configuration.

Since it has been shown (Press and Butler, *loc. cit.*) that the amine reacts with the amino-groups of the nucleic acid, it will necessarily cause a breakage of the hydrogen bonds involving the amino-groups which are alkylated. This is sufficient to account for a loss of viscosity similar in magnitude to that produced by phenol or urea at much higher concentrations. A small number of such acts would be sufficient to reduce considerably the length of the nucleic acid particle. It is possible to understand on this basis the great effect of comparatively small concentrations of the reagent on the physical properties of the nucleic acid. Such a change of the nucleic acid to a more compact configuration would naturally decrease the possibilities of interaction between the nucleic acid particles.

It is evident that even in the phosphate solutions the initial action of the reagent is followed by a continued drift of the sedimentation constants to lower values, which, since it results in lower molecular weights, must be attibuted to a real breakdown of the nucleic acid particles into smaller units. This occurs more quickly and also more extensively in the bicarbonate than in the phosphate solutions and is therefore probably due to a hydrolytic fission of the molecule. The question then arises why nucleic acid which has reacted with the amine is more easily broken down than the original nucleic acid. There are two possible explanations of this behaviour: (1) As suggested by Butler, Gilbert, James, and Ross (*Nature*, 1951, **168**, 985) the tri-esterified phosphate groups, formed by the action of the reagent, are relatively unstable and break down preferentially at the sugar–phosphate bond. The possibility is being investigated further by Dr. W. J. C. Ross. (2) The breakage of the hydrogen bonds between the bases may result in the phosphate–sugar links' being more accessible to attack by hydrolytic reagents.

It will be evident that no simple explanation can be given of the loss of viscosity of nucleic acid solutions when treated with the amine, but that several effects occur each of which is capable of contributing to it. It has been shown that in particular the initial reaction of the reagent brings about (*a*) changes of configuration and (*b*) consequent changes of interaction of the particles with each other, but that at the same time a slow decrease of molecular size of the particles occurs which continues for a considerable time after the initial reaction is completed.

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## 626. *Nitration in Sulphuric Acid. Part VIII.* Kinetics of Nitration of the Trimethylphenylammonium Ion.*

By T. G. Bonner, Freda Bowyer, and Gwyn Williams.

The rates of nitration of the trimethylphenylammonium ion (put in as nitrate or methosulphate) have been measured in 100—82% sulphuric acid solutions. The velocity coefficient for nitration has a maximum value in 90·4% sulphuric acid; and, in a given medium, it varies with initial concentration in a qualitatively predictable manner.

The variation of the nitration velocity coefficient in 90—82% sulphuric acid can be correlated with the carbinol acidity function $J_0$ or directly with results for the extent of ionisation of 4 : 4′ : 4″-trinitrotriphenylcarbinol in the same medium range. The correlation suggests that diminution in the extent of ionisation of nitric acid to nitronium ion is the major factor which causes a 200-fold diminution of the nitration velocity coefficient in this medium range.

The nitration of 2 : 4-dinitrotoluene to 2 : 4 : 6-trinitrotoluene at 60—120° in sulphuric acid–water media (Bennett, Brand, D. M. James, Saunders, and Williams, Part IV, *J.*, 1947, 474) is accompanied by oxidation processes which convert about 40% of the initial nitric acid into nitrous acid at 90°, though without using more than about 3% of the 2 : 4-dinitrotoluene. As shown in Part IV, the nitration reaction can be separated analytically from the simultaneous oxidation, of which the relative extent decreases with falling temperature. Although 2 : 4-dinitrotoluene itself would be nitrated inconveniently slowly at temperatures at which oxidation would be negligible, other compounds can be nitrated at 25° without the complication of a simultaneous oxidation reaction. Detailed experiments have been made with nitrobenzene, *p*-chloronitrobenzene, and the trimethylphenylammonium ion (Bonner, M. E. James, Lowen, and Williams, *Nature*, 1949, **163**, 955). All three compounds are nitrated at an accessible speed at 25° without detectable formation of nitrous acid. The nitration of the trimethylphenylammonium ion has two special features: (*a*) nitration by the nitronium ion is a reaction between two positively charged ions; this circumstance may possibly be expected to give rise to special features in the nitration kinetics; (*b*) the formation of a conjugate acid of the aromatic substance, through proton uptake by the substituent group, is excluded. With some compounds the formation of a conjugate acid may complicate the reaction kinetics in strongly acid media (cf., *e.g.*, Gillespie and Millen, *Quart. Reviews*, 1948, **2**, 277), though, in the light of recent measurements of basic strengths of aromatic nitro-compounds in sulphuric acid media (Brand, *J.*, 1950, 997; Gillespie, *ibid.*, p. 2542), it seems unlikely that formation of the actual conjugate acid can affect the nitration results in 99—85% sulphuric acid, for those nitro-compounds which are nitrated *at measurable speeds* in this medium range, since the extent of their ionisation becomes very small in media containing less than 99% sulphuric acid. However, the results for the nitration of 2 : 4-dinitrotoluene in oleum media (Part IV, *loc. cit.*) require correction for salt formation by the aromatic compound.

The nitration of the trimethylphenylammonium ion has been carried out by using, as starting materials, both trimethylphenylammonium nitrate (the reagents then being automatically present in equivalent concentrations, unless additional nitric acid is added) and (in order to vary initial reagent concentrations independently of one another) trimethylphenylammonium methosulphate in combination with nitric acid. The progress of reaction was followed by analysis for residual nitric acid concentration as a function of time.

The trimethylphenylammonium ion is well known to be nitrated (by concentrated nitric acid at 100°) exclusively in the *m*-position (Vorländer and Siebert, *Ber.*, 1919, **52**, 283). The present work shows that, for given initial reactant concentrations in a specified medium, the rate of nitration (in sulphuric acid at 16—35°) is given by the equation

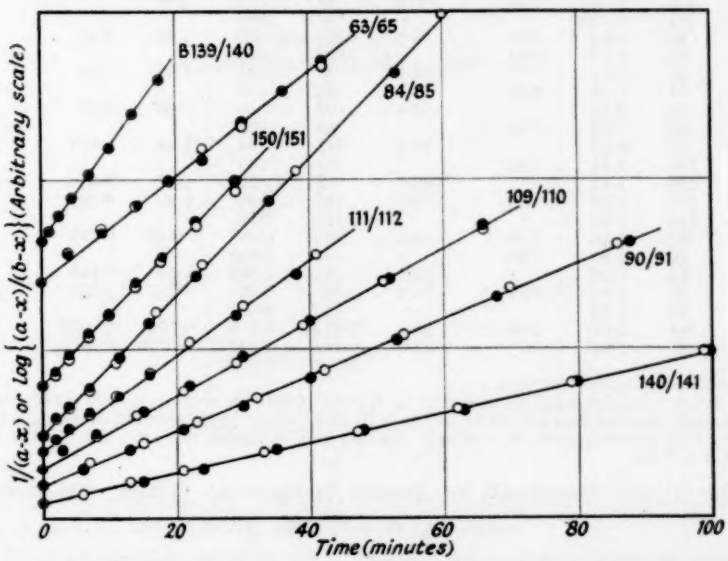
$$-\mathrm{d}[HNO_3]/\mathrm{d}t = k_2[NMe_3Ph^+][HNO_3] \quad . \quad . \quad . \quad . \quad . \quad (1)$$

* Part VII, *J.*, 1950, 3318.

in which $[HNO_3]$ represents the stoicheiometric total concentration of nitric acid. The validity of equation (1) is demonstrated in Fig. 1, which shows linear graphs of $1/[HNO_3]$ plotted against time for reactions in which trimethylphenylammonium nitrate was present alone, and linear graphs of $\log\{[HNO_3]/[NMe_3Ph^+]\}$ for reaction mixtures containing unequal reagent concentrations. The results in Fig. 1 are for different initial conditions, which may be read from the tables; and they also show the reproducibility of duplicate experiments.

*Influence of Medium Composition.*—The results of velocity measurements in different sulphuric acid–water mixtures are shown in Table 1. The general form of the influence of medium composition upon rate of nitration is the same as that observed for the nitration of 2 : 4-dinitrotoluene. At 25°, the nitration of the trimethylphenylammonium ion is fastest in a medium composed of 90·4% of sulphuric acid and 9·6% of water. Nitrobenzene and *p*-chloronitrobenzene have their maximum nitration rate in the same medium at 25° (*Nature*, *loc. cit.*).

FIG. 1. *Course of individual nitrations.*




*Nitration in Media Less Acid than the Optimum.*—For nitric acid, $NO_2{\cdot}OH$, ionising according to equation (2)

$$NO_2{\cdot}OH + 2H_2SO_4 \rightleftharpoons NO_2^+ + OH_3^+ + 2HSO_4^- \quad . \quad . \quad . \quad (2)$$

and for a triarylcarbinol indicator, R·OH, ionising according to a similar equation (cf., *e.g.*, Newman and Deno, *J. Amer. Chem. Soc.*, 1951, **73**, 3644), we may write (Lowen, Murray, and Williams, Part VII, *loc. cit.*) :

$$pK_{NO_2{\cdot}OH} - pK_{R{\cdot}OH} = \log\frac{[NO_2^+]}{[NO_2{\cdot}OH]} - \log\frac{[R^+]}{[R{\cdot}OH]} + \log\left\{\frac{f_{NO_2^+}}{f_{NO_2{\cdot}OH}} \cdot \frac{f_{R{\cdot}OH}}{f_{R^+}}\right\} . \quad (3)$$

in which the $K$'s are thermodynamic equilibrium constants for equilibria of type (2).

If the logarithmic term in activity coefficients in (3) is zero for the medium range considered (see Part VII, *loc. cit.*), then

$$[NO_2^+]/[NO_2{\cdot}OH] = b[R^+]/[R{\cdot}OH] = bR \quad . \quad . \quad . \quad . \quad . \quad (4)$$

in which $b$ is independent of medium composition, and $R = [R^+]/[R{\cdot}OH]$.

An acidity function, $J_0$, for ionisation of type (2), may be defined by equation (5) * (see Part VII, *loc. cit.*) :

$$J_0 = -pK_{NO_2^+\cdot OH} - \log([NO_2^+]/[NO_2\cdot OH]) \quad . \quad . \quad . \quad . \quad (5)$$

or by the equivalent definition $J_0 = H_0 + \log a_{H_2O}$ (Gold and Hawes, *J.*, 1951, 2102), where $H_0$ is Hammett's acidity function.

Then, if the rate or nitration of an aromatic compound is given experimentally

TABLE 1. *Influence of medium composition upon rate of nitration at* 25°

(Initial concentration of $NMe_3Ph^+\}NO_3^- = 0.0252 \pm 0.0002$M.)

| Medium : $H_2SO_4$, wt. % | Expt. no. | $k_2$, min.$^{-1}$ | $k_2$, mean | Medium : $H_2SO_4$, wt. % | Expt. no. | $k_2$, min.$^{-1}$ | $k_2$, mean | $R$ * | $(1 + 0.56R)k_2/R$ |
|---|---|---|---|---|---|---|---|---|---|
| 100 | 24 | 0.51 | 0.51 | 90.8 | 82 | 2.03 | 2.03 | | |
| | 23 | 0.50 | | | 83 | 2.03 | | | |
| 99.6 | 149 | 0.531 | 0.53 | 90.6 | 92 | 2.07 | 2.07 | | |
| | 148 | 0.534 | | | 93 | 2.07 | | | |
| 99.5 | 123 | 0.555 | 0.56 | 90.3 | 84 | 2.06 | 2.06 | | |
| | 124 | 0.555 | | | 85 | 2.06 | | | |
| 98.8 | 68 | 0.64 | 0.64 | 90.1 | 101 | 2.05 | 2.05 | 2.63 | 1.93 |
| 98.3 | 88 | 0.70 | 0.71 | | 102 | 2.05 | | | |
| | 89 | 0.71 | | 89.4 | 86 | 1.91 | 1.92 | 1.78 | 2.17 |
| 97.5 | 91 | 0.81 | 0.82 | | 87 | 1.92 | | | |
| | 90 | 0.82 | | 88.4 | 100 | 1.59 | 1.60 | 1.00 | 2.44 |
| 96.6 | 38 | 0.93 | 0.94 | | 99 | 1.60 | | | |
| | 56 | 0.95 | | 88.3 | 103 | 1.44 | 1.44 | 0.871 | 2.46 |
| 95.6 | 118 | 1.11 | 1.12 | | 104 | 1.44 | | | |
| | 120 | 1.13 | | 87.9 | 72 | 1.27 | 1.27 | 0.646 | 2.68 |
| 94.7 | 117 | 1.27 | 1.30 | 87.5 | 143 | 0.98 | 0.99 | 0.501 | 2.52 |
| | 119 | 1.32 | | | 142 | 0.99 | | | |
| 93.8 | 69 | 1.46 | 1.46 | 86.5 | 140 | 0.444 | 0.446 | 0.219 | 2.29 |
| 91.9 | 70 | 1.86 | 1.88 | | 141 | 0.448 | | | |
| | 73 | 1.90 | | 86.0 | 74 | 0.343 | 0.343 | 0.148 | 2.50 |
| 91.4 | 77 | 1.92 | 1.94 | 85.9 | 162 | 0.286 | 0.287 | 0.132 | 2.33 |
| | 80 | 1.96 | | | 163 | 0.287 | | | |
| 91.0 | 94 | 2.01 | 2.03 | 84.7 | B110 | 0.106 | 0.106 | 0.0575 | 1.90 |
| | 95 | 2.05 | | 82.0 | B111 | 0.0101 | 0.0103 | (0.0078) † | (1.33) † |
| | | | | | B112 | 0.0104 | | | |

* For trinitrotriphenylcarbinol.

† In 82.0% sulphuric acid the indicator ($p$-X·$C_6H_4$)$_3$C·OH (X = $NO_2$) is only 0.78% ionised and the colorimetric measurement of its ionisation is liable to error. Calculated from the $J_0$ function and results for the indicator with X = $NMe_3^+$ (Murray and Williams, *loc. cit.*), $R = 0.0071$ and $(1 + 0.56R)k_2/R = 1.46$.

by equation (1), and theoretically by equation (6), equations (7) hold; and, from (5) and

$$-d[HNO_3]/dt = k_2^\circ[NO_2^+][ArH] \quad . \quad . \quad . \quad . \quad . \quad (6)$$

(7), equation (8) should represent the variation of the experimental velocity coefficient, $k_2$, with medium composition.

$$k_2 = k_2^\circ[NO_2^+]/[HNO_3] = k_2^\circ[NO_2^+]/\{[NO_2^+] + [NO_2\cdot OH]\} \quad . \quad . \quad (7)$$

$$\log k_2 = \log k_2^\circ - pK_{NO_2^+\cdot OH} - J_0 - \log\{1 + [NO_2^+]/[NO_2\cdot OH]\} \quad . \quad . \quad (8)$$

For media in which $[NO_2\cdot OH] \gg [NO_2^+]$, equation (8) reduces to equation (9) :

$$\log k_2 = \log k_2^\circ - pK_{NO_2^+\cdot OH} - J_0 \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

Provided that $k_2^\circ$ of equation (6) does not itself vary with medium, equation (9) predicts that $\log k_2$ should be connected with $J_0$ by a straight line of negative unit gradient.

By this test, equation (9) has been verified in Part VII (*loc. cit.*) for the nitration of the trimethyl-$p$-tolylammonium ion in 76—82% sulphuric acid, whereby the hypothesis of nitration by the $NO_2^+$ ion has been shown to be preferable to that of a nitrating agent (*e.g.*, $H_2NO_3^+$) formed from nitric acid by simple proton uptake (cf. Westheimer and Kharasch, *J. Amer. Chem. Soc.*, 1946, **68**, 1871). In more acid media, the condition $[NO_2\cdot OH] \gg [NO_2^+]$ no longer holds, equation (9) is invalid, and equation (8) must be used.

* $K_{NO_2^+\cdot OH}$ is here defined in terms of the ratio $a_{NO_2^+}/a_{NO_2^+\cdot OH}$ and not of its reciprocal (see Gold and Hawes, *loc. cit.*).

Since the medium range 82—90% sulphuric acid for nitration of the trimethylphenylammonium ion falls within the medium range of variable ionisation of a single indicator, namely 4 : 4′ : 4″-trinitrotriphenylcarbinol (Westheimer and Kharasch, *loc. cit.*; Murray and Williams, *J.*, 1950, 3322; see fifth column of Table 1), it is convenient, instead of (8), to use (10), derived from (7) and (4) :

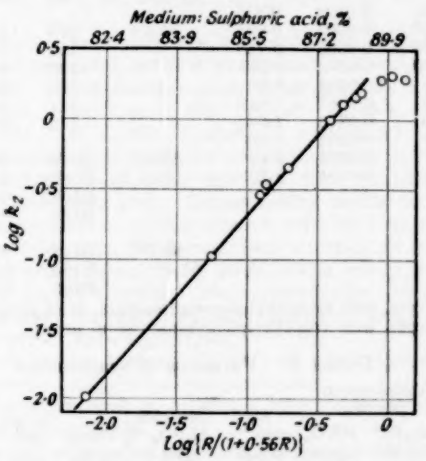
$$\log k_2 = \log k_2^\circ + \log b + \log\{R/(1 + bR)\} \quad . \quad . \quad . \quad . \quad (10)$$

It has been shown previously (Bonner and Williams, *Chem. and Ind.*, 1951, 820) that, for the medium range 86—89·5% sulphuric acid, the results of Table 1 satisfy the equation derived from (1), (6), and (4) :

$$1/R = k_2^\circ b/k_2 - b \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

with the constants $b = 0{\cdot}56$, $k_2^\circ = 4{\cdot}3$ (whence the degree of ionisation of nitric acid to the nitronium ion can be estimated). With this value of $b$, $\log k_2$ is plotted against $\log\{R/(1 + bR)\}$ in Fig 2. The straight line drawn has a gradient of 1·1, which may be compared with the unit gradient demanded by equation (10) if $b$ and $k_2^\circ$, defined by (4) and

FIG. 2.




(6) respectively, remain sensibly constant over the medium range considered. From Fig. 2, the linear relation appears to hold, within experimental error, for the medium range 82·0—88·4% sulphuric acid, covering a 150-fold variation in $k_2$ of equation (1). In only slightly more acid media $k_2$ approaches its maximum value. The linear relation between $\log k_2$ and $J_0$, predicted by equation (9) when ionisation of nitric acid is small, fails in media more acid than 86% sulphuric acid (Part VII, *loc. cit.*). Otherwise expressed, constancy of $b$ and $k_2^\circ$ in equation (10) requires that the ratio $(1 + bR)k_2/R$ should be independent of medium. Col. 6 of Table 1 shows how far this expectation is realised for 90—82% sulphuric acid media, in which $k_2$ changes 200-fold. This more sensitive test indicates that the linear log–log plot in Fig. 2 conceals a small curvature. From carbinol-indicator measurements (Part VII), $b$ is probably constant to within 0·2 log unit over the range 80—85% sulphuric acid. From Fig. 2 and Table 1, it may be provisionally inferred, without excluding the possibility of a change in $k_2^\circ$ by a small multiple, that diminution of the degree of ionisation of nitric acid to the nitronium ion is the major factor which reduces $k_2$ by 200-fold in the medium range 90—82% sulphuric acid.

*Nitration in Media More Acid than the Optimum.*—The variation in $k_2$ over the medium range 100—90% sulphuric acid cannot at present be treated quantitatively (cf. Hughes, Ingold, and Reed, *J.*, 1950, 2400). The absence of an isotope effect in aromatic nitration (Melander, *Nature*, 1949, **163**, 499; *Arkiv Kemi*, 1950, **2**, 211), recently confirmed (unpublished experiments) under the conditions of kinetic experiments for nitrobenzene

and deuteronitrobenzene, is evidence against the hypothesis of basic catalysis by bisulphate ion (Part IV, *loc. cit.*).

*Influence of Reagent Concentrations.*—Increase of initial concentration of either reagent causes increase of anion concentration in the system. From the observed influence of water upon the nitration rate (Table 1), it may therefore be expected that rise of initial reagent concentration should increase or decrease $k_2$ in media more acid or less acid respectively than the optimum. Table 2 shows that these expectations are realised

TABLE 2. *Variation of initial reactant concentrations at 25°.*

| Medium: $H_2SO_4$, % | Expt. no. | Initial concn. (M) of: $NMe_2Ph^+$ | $HNO_3$ | $k_2$, min.$^{-1}$ | Medium: $H_2SO_4$, % | Expt. no. | Initial concn. (M) of: $NMe_2Ph^+$ | $HNO_3$ | $k_2$, min.$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| 100 * | B182 | 0·0502 | 0·0301 | 0·483 | 96·6 † | 38 | 0·0251 | 0·0251 | 0·93 |
| | B183 | 0·0502 | 0·0301 | 0·482 | | 56 | 0·0251 | 0·0251 | 0·95 |
| | B184 | 0·300 | 0·0300 | 0·728 | | 60 | 0·0253 | 0·0503 | 0·99 |
| | B185 | 0·300 | 0·0302 | 0·725 | | 64 | 0·0254 | 0·0755 | 0·98 |
| | B186 | 0·500 | 0·0300 | 0·843 | | 63 | 0·0255 | 0·1004 | 0·95 |
| | B187 | 0·500 | 0·0302 | 0·873 | | 65 | 0·0251 | 0·1003 | 0·95 |
| | B214 | 2·457 | 0·0299 | 0·712 | | 58 | 0·0504 | 0·0504 | 1·00 |
| | | | | | | 34 | 0·0501 | 0·0501 | 1·00 |
| 97·4 * | B137 | 0·0495 | 0·0298 | 0·835 | | | | | |
| | B138 | 0·0500 | 0·0303 | 0·822 | 88·3 † | 103 | 0·0252 | 0·0252 | 1·44 |
| | B139 | 0·1003 | 0·0305 | 0·915 | | 104 | 0·0253 | 0·0253 | 1·44 |
| | B140 | 0·1000 | 0·0305 | 0·911 | | 113 | 0·0252 | 0·0485 | 1·42 |
| | B141 | 0·200 | 0·0304 | 1·00 | | 114 | 0·0250 | 0·0484 | 1·45 |
| | B142 | 0·200 | 0·0301 | 1·01 | | 111 | 0·0504 | 0·0504 | 1·41 |
| | B143 | 0·300 | 0·0303 | 1·06 | | 112 | 0·0504 | 0·0504 | 1·39 |
| | B144 | 0·300 | 0·0302 | 1·08 | | | | | |
| | B145 | 0·401 | 0·0306 | 1·19 | 88·2 * | B170 | 0·0504 | 0·0312 | 1·33 |
| | B146 | 0·401 | 0·0304 | 1·22 | | B180 | 0·0503 | 0·0310 | 1·36 |
| | | | | | | B168 | 0·302 | 0·0312 | 0·518 |
| | | | | | | B169 | 0·302 | 0·0312 | 0·510 |
| | | | | | 86·7 * | B162 | 0·0499 | 0·0301 | 0·414 |
| | | | | | | B164 | 0·0503 | 0·0302 | 0·410 |
| | | | | | | B163 | 0·0998 | 0·0302 | 0·298 |

* Expts. with trimethylphenylammonium methosulphate.
† Expts. with trimethylphenylammonium nitrate.

TABLE 3. *Variation of temperature.*

| Medium: $H_2SO_4$, % | Temp. | Expt. no. | Initial concn. (M) of: $NMe_2Ph^+$ | $HNO_3$ | $k_2$, min.$^{-1}$ | Medium: $H_2SO_4$, % | Temp. | Expt. no. | Initial concn. (M) of: $NMe_2Ph^+$ | $HNO_3$ | $k_2$, min.$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 100 * | 15° | B250 | 0·0501 | 0·0300 | 0·209 | 99·6 † | 15·92° | 154 | 0·0252 | 0·0252 | 0·225 |
| | 25 | B251 | 0·0500 | 0·0300 | 0·557 | | 15·92 | 155 | 0·0252 | 0·0252 | 0·227 |
| | 35 | B249 | 0·0503 | 0·0298 | 1·25 | | 19·97 | 158 | 0·0253 | 0·0253 | 0·346 |
| | | | | | | | 19·97 | 159 | 0·0252 | 0·0252 | 0·344 |
| 87·5 † | 15·92° | 156 | 0·0254 | 0·0254 | 0·479 | | 24·96 | Mean from Table 1 | | | 0·533 |
| | 15·92 | 157 | 0·0252 | 0·0252 | 0·481 | | 29·96 | 160 | 0·0254 | 0·0254 | 0·821 |
| | 24·96 | Mean from Table 1 | | | 0·985 | | 29·96 | 161 | 0·0252 | 0·0252 | 0·821 |
| | 35·00 | 150 | 0·0254 | 0·0254 | 2·01 | | 35·00 | 152 | 0·0252 | 0·0252 | 1·21 |
| | 35·00 | 151 | 0·0252 | 0·0252 | 2·05 | | 35·00 | 153 | 0·0252 | 0·0252 | 1·21 |

* Expts. with trimethylphenylammonium methosulphate.
† Expts. with trimethylphenylammonium nitrate.

qualitatively. The effects are barely accessible to experiments with trimethylphenylammonium nitrate, because initial heat effects (see Experimental section) forbid any substantial increase of initial concentration; but they are seen clearly in experiments with the methosulphate. Quantitative aspects will be considered in later papers, together with results for other compounds. In media more aqueous than the optimum for nitration, the addition of various substances to the medium produces an apparent lowering of the (colorimetric) extent of ionisation of a triarylcarbinol indicator and thus (if the effect is real) should affect the extent of ionisation of nitric acid to nitronium ion. The effects of added substances upon the apparent ionisation of an indicator can be correlated with their effects upon speed of nitration (unpublished experiments).

The effects of adding potassium hydrogen sulphate to reaction mixtures resemble (qualitatively at least) those of change of initial concentration of the aromatic salt.

*Influence of Temperature.*—Results at different temperatures are shown in Table 3. Over the small temperature range of 20°, the graph of log $k_2$, plotted against $1/T$, is linear for 87·5% sulphuric acid medium and corresponds to $k_2 = 1{\cdot}05 \times 10^8$ exp.$-13{,}390/RT$ litre mole$^{-1}$ sec.$^{-1}$. The results for 99·6% sulphuric acid medium correspond roughly to the equation $k_2 = 1{\cdot}4 \times 10^9$ exp.$-15{,}300/RT$ litre mole$^{-1}$ sec.$^{-1}$; but the graph of log $k_2$ against $1/T$ shows a distinct curvature, giving a maximum range for $E$ of 14·9—16·3 kcal. In 100% sulphuric acid $E$ appears to range from 14·7 ± 0·6 to 16·8 ± 0·6 kcal. at 15—35°.

## Experimental

*Materials.*—Trimethylphenylammonium iodide (made in 96% yield from methyl iodide and dimethylaniline, m. p. after recrystallisation from absolute alcohol 216—218°, decomp.) was converted into the nitrate by the action of aqueous silver nitrate, excess of silver being removed with hydrogen sulphide. The nitrate, obtained by evaporation to dryness, was recrystallised from a solution in the minimum quantity of hot absolute alcohol to which was then added about twelve times the volume of hot dry acetone. The nitrate separated on cooling; m. p. 119—119·5° (Found: C, 54·2; H, 7·1; N, 14·0. Calc. for $C_9H_{14}O_3N_2$: C, 54·5; H, 7·1; N, 14·1%). Vorländer and Siebert (*Ber.*, 1919, **52**, 283) give m. p. 110—115° for the nitrate recrystallised from alcohol–ether.

Trimethylphenylammonium methosulphate was made from dimethylaniline and dimethyl sulphate in dry benzene; it separated out as it was formed, and was recrystallised from a small amount of absolute alcohol. Crystallisation could be accelerated, if desired, by addition of a large volume of dry acetone. It had m. p. 121° (Found: C, 49·1, 49·0; H, 7·2, 7·1; N, 5·5, 5·5; S, 12·8, 12·6. Calc. for $C_{10}H_{17}O_4NS$: C, 48·6; H, 6·9; N, 5·7; S, 13·0%).

Pure nitric acid and other inorganic materials were obtained as described in Part IV (*loc. cit.*).

*Nitration Media.*—Sulphuric acid–water mixtures were prepared by adding water or redistilled oleum to 98% sulphuric acid. Corresponding media containing nitric acid were obtained by addition of anhydrous nitric acid (freshly distilled from sulphuric acid). Analysis was by alkali titration coupled with ferrous sulphate titration of nitric acid. For example, a medium was found to contain 96·45, 96·56, 96·60, 96·58, mean 96·55%, of sulphuric acid, giving the mol. ratio $H_2O/H_2SO_4 = 0{\cdot}195$. The corresponding nitric acid solution contained total acid 0·01962, 0·01959, 0·01959, 0·01958 equiv./g., and nitric acid 4153, 4145, 4137 × 10$^{-7}$ mole/g., giving mol. ratio $H_2O/H_2SO_4 = 0{\cdot}193$.

Alkali was standardised against 1N-hydrochloric acid, itself standardised gravimetrically or referred to standard potassium iodate or sodium carbonate.

100% Sulphuric acid was prepared by adjusting 98% sulphuric acid to maximum freezing point by the addition of sulphur trioxide.

*Velocity Measurements.*—Temperature was controlled to ±0·02° and measured on an N.P.L. calibrated thermometer. Most of the velocity measurements were made at 25° (more exactly 24·96°). Solutions were made up to known volumes at thermostat temperature and combined in such a way that the weight of each component of the reaction mixture (including weight of solvent) was always known. The reaction vessel was a three-necked flask carrying a thermometer and a mercury-sealed electric stirrer. Before transference to this vessel, the reagents were pre-mixed in a flask with standard socket neck by addition of the second component from a container with standard cone neck.

When trimethylphenylammonium nitrate was used, 0·245—0·250 g. of the solid salt was added to 50 ml. of medium in the pre-mixing flask, thus starting the nitration. When required, nitric acid was incorporated in the medium to give a higher initial concentration than that provided by the salt alone. With trimethylphenylammonium methosulphate, either 25 ml. of nitric solution were added to 25 ml. of salt solution, or, later, a small volume of more concentrated nitric acid solution was added to 50 ml. of medium containing the aromatic salt and, sometimes, an added inorganic salt or other compound when the effects of these upon nitration rate were to be investigated.

The starting time of the reaction was always taken as the instant at which the reagents came together. Pre-mixing and transfer to the reaction vessel (with stirrer already in operation) required 40—50 seconds with the nitrate and 10—15 seconds with the methosulphate.

The temperature rose by 0·4—0·7° when the solid nitrate salt was added to sulphuric acid; and the temperature of the reaction mixture was observed at one-minute intervals. Normally, the temperature of the reaction mixture came to within 0·05° of that of the thermostat in about 5 minutes and to within 0·02° in about 8 minutes. The total time of observation for a reaction

varied from 20 to 240 minutes, being frequently of the order 100 minutes. From the known activation energies, the errors in the velocity coefficients caused by the initial temperature fluctuations were calculated to be not more than 1%. Thermal disturbances were minimised by working with only 0·025M-reagents; and initial errors of all kinds were eliminated as far as possible by evaluating velocity coefficients graphically. Initial temperature rise in the reaction mixture was always much less when it was prepared from the solution of the methosulphate salt.

*Analysis of Reaction Mixtures.*—5-Ml. samples were extracted at intervals in pipettes calibrated to deliver the reaction media employed with a standard delivery time, including drainage, of 25 sec. (*e.g.*, a pipette delivered 4·92 ± 0·02 ml. for 87·8—100% sulphuric acid). The samples were run into 30 ml. of ice-cold concentrated sulphuric acid, and their nitric acid content was determined by titration against M/20-ferrous sulphate solution in 40% sulphuric acid (preserved under nitrogen), Treadwell and Vontobel's potentiometric method (*Helv. Chim. Acta*, 1937, **20**, 573; cf. Part IV) being used. The titration was carried out under nitrogen, with mechanical stirring. The ferrous sulphate solution was run in from a 10-ml. micro-burette, and the end-point of the titration was marked by a sharp increase of potential of 200—300 mv for the addition of 0·02—0·03 ml. of reagent, provided that the sulphuric acid content at the end of the titration lay between 80 and 95%. This was ensured by adjustment, when necessary. Standardisation of a ferrous sulphate solution against (*a*) potassium nitrate dissolved in 96% sulphuric acid, (*b*) aqueous potassium nitrate, and (*c*) potassium permanganate, gave its concentration as (*a*) 0·0504, (*b*) 0·0508, (*c*) 0·0505N.

Some preliminary experiments were made on the reverse method of running nitrate into excess of standard ferrous sulphate, and observing the potentiometric end-point. The following was a test of the reverse titration: 5-ml. portions of approximately M/5-ferrous sulphate solution in 40% sulphuric acid were run into 40 ml. of concentrated sulphuric acid and titrated by running in M/10-potassium nitrate solution (in 40% sulphuric acid) from the burette to the end-point, which was marked by a drop in potential of about 150 mv, not quite so sharply defined as in the commoner forward titration. Volumes required were: 4·71, 4·70, 4·65, 4·66, 4·76, 4·63, 4·65, 4·68, mean 4·68 ml. From these results, 1 ml. of the potassium nitrate solution was equivalent to 1·07 ml. of ferrous sulphate solution, which may be compared with 1·06 ml. obtained by the forward titration. The reverse titration was not used for velocity measurements.

*Absence of Nitrous Acid in Reaction Products.*—2-Ml. samples of reaction mixture containing initially 0·025M-trimethylphenylammonium nitrate were run into 10 ml. of approx. N/20-chloramine-T solution, diluted to 100 ml.; and the solutions were titrated, by use of potassium iodide and sodium thiosulphate, with the following results:

(i) Medium: 87·5% sulphuric acid.

| Time (min.) | 14 | 23 | 34 | 45 | 60 | 78 |
|---|---|---|---|---|---|---|
| Titre (ml.) | 11·71 | 11·79 | 11·79 | 11·76 | 11·70 | 11·68 |

(ii) Medium: 99·6% sulphuric acid.

| Time (min.) | 15 | 41 | 70 | 92 | 110 |
|---|---|---|---|---|---|
| Titre (ml.) | 11·76 | 11·71 | 11·71 | 11·75 | 11·72 |

The blank titration for 10 ml. of chloramine-T solution after addition of 2 ml. of sulphuric acid, containing no aromatic nitrate, was 11·70 ml. Evidently, no nitrous acid is formed in the reaction.

*Results.*—Unless otherwise stated, times are given in minutes. One experiment is given in full below.

*Expt.* 91. Temp. 25·0°. Initial concn. of nitrate salt = 0·0254M. Medium: 97·5% $H_2SO_4$.

| Time, min. | 0 | 6 | 13 | 21 | 30 | 40 | 53 | 68 | 88 |
|---|---|---|---|---|---|---|---|---|---|
| Concn. of $HNO_3$, M | 0·0254 | 0·0223 | 0·0199 | 0·0177 | 0·0158 | 0·0139 | 0·0122 | 0·01055 | 0·0091 |
| Change, % | — | 12·2 | 21·8 | 30·4 | 37·8 | 45·1 | 52·0 | 58·5 | 64·5 |
| $k_2$ | — | 0·91 | 0·84 | 0·82 | 0·80 | 0·81 | 0·81 | 0·82 | 0·81 |

$k_2$ from linear plot (Fig. 1) = 0·81.

The reproducibility of the velocity measurements may be judged from Fig. 1.

The investigation of this compound was originally suggested by Dr. G. M. Bennett, C.B., F.R.S. We wish to acknowledge that most of the experimental work was done (in 1946—1948) while one of us (T. G. B.) held a Further Education Grant from the Ministry of Education. We thank Imperial Chemical Industries Limited for financial help.

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## 627. *Syntheses of Benzo*[mno]*fluoranthene and Dibenzo*[k, mno]-*fluoranthene.**

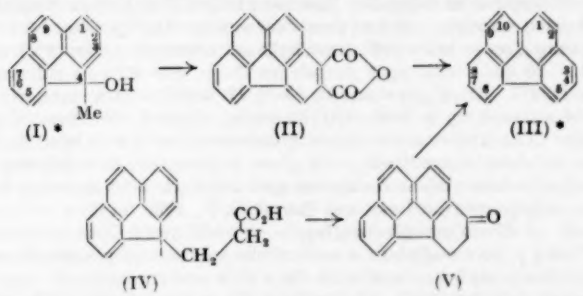
By Neil Campbell and David H. Reid.

The syntheses of benzo[*mno*]fluoranthene (III) and dibenzo[*k*, *mno*]fluoranthene (VIII) are described. These are the first examples known of hydrocarbons having four aromatic nuclei fused directly to a *cyclo*pentane ring.

So far as we are aware no hydrocarbon with four aromatic nuclei annealed directly to a *cyclo*pentane ring has hitherto been synthesised. We have synthesised two hydrocarbons of this type, namely, benzo[*mno*]fluoranthene * (2 methods) and dibenzo[*k*, *mno*]fluoranthene.*

4-Keto*cyclo*penta[*def*]phenanthrene * and methylmagnesium iodide gave the carbinol (I) which, when heated with maleic anhydride and acetic anhydride, underwent a Diels–Alder reaction to form benzo[*mno*]fluoranthene-3 : 4-dicarboxylic acid anhydride (II) (cf. Campbell and Wang, *J.*, 1949, 1513; Campbell and Gow, *J.*, 1949, 1555). The yield of anhydride was poor owing partly at least to extensive polymerisation. It is noteworthy that the tetrahydro-product initially formed was not isolated, but had been transformed into the more stable, fully aromatic compound. Decarboxylation of the anhydride gave benzo[*mno*]fluoranthene (III).

In the second synthesis reaction of vinyl cyanide with methyl 4-*cyclo*penta[*def*]phenanthrene-4-carboxylate (cf. Tucker and Campbell, *J.*, 1949, 2623) gave a nitrile which was hydrolysed to 4-2′-carboxyethyl-4-*cyclo*penta[*def*]phenanthrene (IV). This was cyclised to 3 : 4 : 5 : 5*a*-tetrahydro-3-ketobenzo[*mno*]fluoranthene (V), the cyclisation proceeding as easily as that of β-9-fluorenylpropionic acid (Campbell and Tucker, *loc. cit.*). Reduction of the ketone to the tetrahydrobenzofluoranthene and subsequent dehydrogenation gave benzo[*mno*]fluoranthene (III), identical with the product of the first synthesis.



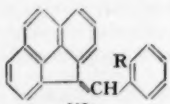

(I) * (II) (III) * (IV) (V)

4-*cyclo*Penta[*def*]phenanthrene-4-carboxylic acid was prepared by condensing 4-*cyclo*penta[*def*]phenanthrene with methyl oxalate, followed by hydrolysis and oxidation of the resulting methyl 4-*cyclo*penta[*def*]phenanthrene-4-glyoxylate. The acid had the same m. p. as that prepared in a different way by Kruber (*Ber.*, 1934, **67**, 1000). The methyl ester was obtained in tautomeric forms : the keto-form, formed very pale yellow needles, m. p. 129°, and the enolic form bright yellow needles, m. p. 122°. The tautomerism was established by the preparation of a 2 : 4-dinitrophenylhydrazone and a benzoate. Analogous instances of tautomerism are found in ethyl 9-fluorenylglyoxylate (Kuhn and Levy, 1928, **61**, 2240) and ethyl *o*-fluorenylformylbenzoate (Wislicenus and Neber, *Annalen*, 1919, **418**, 274).

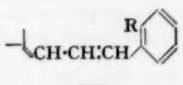
A related hydrocarbon, dibenzo[*k*, *mno*]fluoranthene * (VIII) was also synthesised. 4-*cyclo*Penta[*def*]phenanthrene condensed with *o*-chlorobenzaldehyde to give two products : the *o*-chlorobenzylidene derivative (VI, R = Cl) and a deep yellow substance. The latter is probably a diene (VII, R = Cl) formed from acetaldehyde which is obtained from the

* (I), (III), and (VIII), and their derivatives, are named and numbered in accordance with Ring Index principles; (I), cf. R.I. 2570.

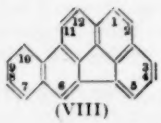
sodium ethoxide used as condensing agent (cf. De Fazi, *Gazzetta*, 1921, **51**, 328; Bergmann, *Ber.*, 1930, **63**, 1617, 2598; Rieveschl and Ray, *Chem. Reviews*, 1938, **23**, 314). Cyclisation of (VI) with a boiling concentrated solution of potassium hydroxide in quinoline gave the dibenzofluoranthene (VIII). A better yield was obtained when the bromobenzylidene compound (VI, R = Br) was used.



(VI)



(VII)



(VIII)

## Experimental

Adsorptions were effected on alumina (Brockmann) and, unless otherwise stated, fluorescence observations were made in ultra-violet light from a Hanovia lamp. Analyses were by Drs. Weiler and Strauss, Oxford.

*First Synthesis of Benzo*[mno]*fluoranthene.*—4-Keto*cyclo*penta[*def*]phenanthrene (0·75 g.) in ether (4 c.c.) and benzene (6 c.c.) was added to the Grignard reagent prepared from magnesium (0·13 g.) and methyl iodide (0·768 g.) in ether (3 c.c.). The solution was boiled for 40 minutes and was then poured on crushed ice (2·0 g.). Working up the mixture in the usual manner gave 4-*hydroxy*-4-*methyl*cyclo*penta*[def]*phenanthrene* (I) (0·615 g.) as prisms (from benzene or by sublimation) (Found: C, 86·9; H, 5·7. $C_{16}H_{12}O$ requires C, 87·2; H, 5·5%). The carbinol has a blue fluorescence both in the solid state and in benzene. With concentrated sulphuric acid it gives a green colour.

The carbinol (0·812 g.), maleic anhydride (3·6 g.), and acetic anhydride (12 c.c.) were boiled for 1 hour. The cooled solution overnight deposited *benzo*[mno]*fluoranthene*-3 : 4-*dicarboxylic acid anhydride* (II) (39 mg.), which on sublimation gave orange-yellow needles, m. p. 335—340° (decomp.) on slow heating (Found: C, 81·1; H, 2·9. $C_{20}H_8O_3$ requires C, 81·1; H, 2·7%). The anhydride gives a green colour in concentrated sulphuric acid and has an intense yellowish-green fluorescence in benzene or ethanol. The anhydride (19 mg.) was intimately mixed with lime (4·0 g.) and heated strongly. *Benzo*[mno]*fluoranthene* (III) distilled off and was obtained in yellow needles (4 mg.), m. p. 147—149°, from light petroleum (b. p. 60—80°), after purification on a column (0·6 × 20 cm.) with light petroleum (b. p. 60—80°) as solvent and developer (Found: C, 95·2; H, 4·7. $C_{18}H_{10}$ requires C, 95·5; H, 4·5%). It forms a *dipicrate* as golden-yellow needles (from ethanol), m. p. 205—210° (decomp.) (Found: N, 12·3. $C_{18}H_{10}.2C_6H_3O_7N_3$ requires N, 12·3%). The hydrocarbon has a greenish-yellow and a blue fluorescence in the solid state and in solution, respectively. It gives a green colour with warm concentrated sulphuric acid, and with benzylidene dichloride and sulphuric acid affords a heliotrope colour which changes to a deep purple (cf. Levy and Campbell, *J.*, 1939, 1442).

*Second Synthesis of Benzo*[mno]*fluoranthene.*—4-*cyclo*Penta[*def*]phenanthrene (5·7 g.) and methyl oxalate (7·09 g.) were added to a methanolic solution of potassium methoxide [from potassium (2·35 g.) and anhydrous methanol (24 c.c.)], and the mixture was warmed until a homogeneous solution was obtained. The bulk of the methanol was slowly distilled off, and the remaining methanol and methyl oxalate were removed by distillation at 100°/10 mm. The residual methyl 4-*cyclo*penta[*def*]phenanthrene-4-glyoxylate was hydrolysed with boiling glacial acetic acid (40 c.c.), concentrated sulphuric acid (5 c.c.), and water (20 c.c.) for 60 minutes. The solution was poured into water (200 c.c.) and gave the *glyoxylic acid* (8·6 g.) as greenish-yellow needles (from acetic acid), m. p. 231—234° (decomp.) (Found: C, 71·1; H, 4·4. $C_{17}H_{10}O_3.C_2H_4O_2$ requires C, 70·8; H, 4·4%). 30% Hydrogen peroxide (1 c.c.) was added to the keto-acid (0·2 g.) in glacial acetic acid (2 c.c.) and the mixture kept for 120 hours. The yellow precipitate became straw-coloured, and on crystallisation from ethanol gave 4-*cyclo*penta[*def*]phenanthrene-4-carboxylic acid as needles, m. p. 251—252° (decomp.).

Dry hydrogen chloride was passed into a suspension of the acid (1·10 g.) in absolute methanol (80 c.c.) until the weight of the mixture had increased by 3 g. The clear solution was boiled (12 hours) and methanol (50 c.c.) then removed by distillation. The residual liquid was poured into water (100 c.c.), and the resulting emulsion was extracted several times with benzene until the aqueous layer was clear. The combined benzene extracts were washed first with dilute aqueous sodium carbonate and then with water, and were finally dried ($Na_2SO_4$). Removal of the benzene yielded an oil which was dissolved in boiling light petroleum (b. p. 80—100°). The solution was cooled and immediately it became turbid it was placed in a bath of acetone–solid

carbon dioxide. The *methyl* ester (0·8 g.) was deposited as needles, m. p. 61—62° (Found: C, 81·5; H, 5·1. $C_{17}H_{12}O_2$ requires C, 82·2; H, 4·9%). The sodium carbonate solution on acidification deposited unchanged acid (90 mg.).

Vinyl cyanide (0·22 c.c.) was added to the ester (0·79 g.) in 2-ethoxyethanol (3 c.c.) containing potassium hydroxide (11·2 g./litre of solution). After 1 hour 2-ethoxyethanol (2 c.c.) and 10N-potassium hydroxide (4·5 c.c.) were added and the mixture boiled for 1 hour. The mixture was then added to water (50 c.c.), and the solution boiled with charcoal and filtered. Acidification gave 4-2′-*carboxyethyl*-4-cyclo*penta*[def]*phenanthrene* (IV) (0·75 g.), as needles (from benzene–acetone; 4 : 1 by vol.), m. p. 149—153° (Found: C, 80·9; H, 5·6. $C_{19}H_{14}O_2$ requires C, 82·4; H, 5·4%).

The acid (0·72 g.) was converted into the chloride by dissolving it in anhydrous benzene (4 c.c.) and adding phosphorus pentachloride (0·63 g., 10% excess). After 30 minutes the solution was boiled for 5 minutes and then cooled, and stannic chloride (0·64 c.c.; 100% excess) added. The mixture was kept for 40 minutes and then poured into concentrated hydrochloric acid (30 c.c.); the resultant mixture was extracted with benzene (40 c.c.) and chloroform (10 c.c.), and the extract washed twice with dilute sodium carbonate solution and then evaporated. The oily residue was dissolved in light petroleum (b. p. 80—100°) containing a little benzene. Evaporation to a small volume gave 3 : 4 : 5 : 5a-*tetrahydro*-3-*ketobenzo*[mno]*fluoranthene* (V) (52%), as pale yellow plates (from light petroleum–benzene; 3 : 1 by vol.), m. p. 159—161° (Found: C, 87·7; H, 5·0. $C_{18}H_{12}O$ requires C, 88·5; H, 5·0%). It affords a 2 : 4-*dinitrophenylhydrazone*, as orange-red needles (from glacial acetic acid), m. p. 293—296° (sublimes and decomp.) (Found: N, 13·4. $C_{24}H_{16}O_4N_4$ requires N, 13·2%). With concentrated sulphuric acid the ketone gives a bright yellow solution with a greenish-yellow fluorescence. The ketone (0·33 g.) in toluene (4 c.c.) was boiled for 60 hours with concentrated hydrochloric acid (4 c.c.), water (6 c.c.), acetic acid (2 c.c.), and 10% zinc amalgam (2 g.), concentrated hydrochloric acid (2 c.c. portions) being added after 6 and 18 hours. The toluene layer was washed with dilute sodium carbonate solution and then with water. The dried extract ($Na_2SO_4$) gave on evaporation 3 : 4 : 5 :5a-*tetrahydrobenzo*[mno]*fluoranthene* as plates (from ethanol), m. p. 78—79·5° (Found: C, 93·6; H, 6·1. $C_{18}H_{14}$ requires C, 93·9; H, 6·1%). The hydrocarbon was heated for 90 minutes at 300° with 20% palladium–charcoal, and the product extracted with chloroform. Evaporation of the solvent gave benzo[*mno*]fluoranthene (113 mg.) as yellow needles (from light petroleum), m. p. 145—148°, showing no depression in melting point when mixed with the hydrocarbon prepared as above.

*Properties of Methyl* 4-cyclo*Penta*[def]*phenanthrene*-4-*glyoxylate*.—The crude ester from 4-*cyclo*penta[*def*]phenanthrene (1·90 g.) and methyl oxalate (2·36 g.) was dissolved in warm acetic acid (12 c.c.) and precipitated with concentrated sulphuric acid (2 c.c.) in water (8 c.c.). The precipitate was washed with water (100 c.c.), dried, and crystallised from light petroleum (b. p. 60—80°), giving *methyl* 4-cyclo*penta*[def]*phenanthrene*-4-*glyoxylate* (73%), as bright yellow plates which partially melt at 122°, then giving almost colourless needles which completely melted by 129° (Found: C, 78·3; H, 4·4. $C_{19}H_{12}O_3$ requires C, 78·3; H, 4·4%). In one experiment crystallisation from light petroleum–acetone gave very pale yellow needles, m. p. 129° (sharp). The ester forms a 2 : 4-*dinitrophenylhydrazone*, as yellow needles (from acetic acid), m. p. 240—243° (Found: N, 12·2. $C_{24}H_{16}O_6N_4$ requires N, 12·3%), and with sodium ethoxide in ethanol and subsequent addition of benzoyl chloride yields a *benzoate*, as yellow plates (from ethanol), m. p. 167—168° (Found: C, 78·5; H, 4·3. $C_{25}H_{16}O_4$ requires C, 79·0; H, 4·3%).

The acid (0·40 g.) was boiled with toluene (10 c.c.), concentrated hydrochloric acid (6 c.c.), water (2 c.c.), glacial acetic acid (3 c.c.), and 10% zinc amalgam (2 g.) for 60 hours, hydrochloric acid (2 c.c. portions) being added after 6 and 18 hours. The toluene layer was washed free from mineral acid with water, and the organic acid was then extracted with dilute sodium hydroxide solution (3 × 25 c.c.). Precipitation with hydrochloric acid afforded 4-(*carboxyhydroxymethyl*)-4-cyclo*penta*[def]*phenanthrene* (0·32 g.) as needles (from benzene), m. p. 211—212° (sublimes) (Found: C, 77·3; H, 4·3. $C_{17}H_{12}O_3$ requires C, 77·3; H, 4·6%).

*Synthesis of Dibenzo*[k, mno]*fluoranthene*.—4-*cyclo*Penta[*def*]phenanthrene (0·452 g.) was added to an ethanolic solution of sodium ethoxide [from sodium (0·081 g.) and absolute ethanol (5 c.c.)], and the temperature was raised to 75°. This temperature was maintained while a solution of *o*-chlorobenzaldehyde (0·335 g.) in absolute ethanol (2 c.c.) was added dropwise. The solution was boiled for 30 minutes, cooled, and poured into water (50 c.c.). The mixture was extracted with benzene (50 c.c.), and the dried extract ($Na_2SO_4$) on evaporation gave 4-*o*-*chlorobenzylidene*cyclo*penta*[def]*phenanthrene* (VI, R = Cl), as pale lime-green needles [from

light petroleum (b. p. 60—80°)], m. p. 136—137° (Found: C, 84·2; H, 4·3; Cl, 11·4. $C_{22}H_{13}Cl$ requires C, 84·5; H, 4·2; Cl, 11·3%). The presence of a double bond was shown by the usual tests. The combined filtrates were passed through a column (20 × 2·2 cm.). Development with light petroleum gave 2 bands; a lower lime-green band with a yellow fluorescence, which yielded the above chlorobenzylidene compound (0·614 g.), m. p. 136—137°, and a upper, bright yellow zone with a yellow fluorescence which afforded 4-3′-*o*-*chlorophenylallylidene*cyclo*penta*-[def]*phenanthrene* (VII, R = Cl) (51 mg.), m. p. 182—184° (Found: C, 84·4; H, 4·4; Cl, 11·2. $C_{24}H_{15}Cl$ requires C, 85·1; H, 4·5; Cl, 10·5%). It gives a green colour with sulphuric acid.

The *o*-chlorobenzylidene compound (0·27 g.) was boiled for 90 minutes in quinoline (2 c.c.) with potassium hydroxide (0·78 g.), and the mixture poured into concentrated hydrochloric acid (50 c.c.). The acid mixture was extracted with benzene (5 × 100 c.c.), and the extract washed successively with water, dilute sodium carbonate, and water. The dried solution ($Na_2SO_4$) was concentrated to 40 c.c. and passed through a column (20 × 2·1 cm.). Development with light petroleum (b. p. 60—80°) gave a pale yellow zone with a purple fluorescence from which *dibenzo*-[k, mno]*fluoranthene* (35%), m. p. 147—149°, was obtained as pale green needles (from light petroleum) (Found: C, 95·0; H, 4·8. $C_{22}H_{12}$ requires C, 95·6; H, 4·4%). The *picrate* dissociates easily and could not be obtained pure; it was obtained as orange-red needles, m. p. 165—175° (decomp.) (Found: N, 9·8. $C_{28}H_{15}O_7N_3$ requires N, 8·3%). The *trinitrobenzene* compound separated from ethanol in yellow needles, m. p. 170—175° (decomp.) (Found: N, 8·8. $C_{28}H_{15}O_6N_3$ requires N, 8·6%). The hydrocarbon in solution has a strong purple fluorescence both in daylight and in ultra-violet light. With warm concentrated sulphuric acid it gives a green colour which changes to olive-green and finally to brown. Benzylidene dichloride, when added to a suspension of the hydrocarbon in concentrated sulphuric acid, gives a blue colour. The hydrocarbon does not form an adduct with maleic anhydride.

A similar series of reactions gave the following compounds: 4-*o*-*Bromobenzylidene*cyclo*penta*[def]*phenanthrene* (VI, R = Br) (66%) as lime-green needles (from light petroleum), m. p. 147—149° (Found: C, 73·7; H, 3·5; Br, 21·8. $C_{22}H_{13}Br$ requires C, 74·0; H, 3·7; Br, 22·4%), which in the solid state has a yellow-green and in solution a bluish-green fluorescence. 4-3′-*o*-*Bromophenylallylidene*cyclo*penta*[def]*phenanthrene* (VII, R = Br) as yellow prisms (from light petroleum), m. p. 198—200° (Found: C, 75·1; H, 3·6; Br, 20·8. $C_{24}H_{15}Br$ requires C, 75·2; H, 4·0; Br, 20·8%); this has a yellow fluorescence in the solid state and an intense yellowish-green fluorescence in solution. The bromobenzylidene compound with potassium hydroxide and quinoline gave dibenzo[*k*, *mno*]fluoranthene in 50% yield.

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UNIVERSITY OF EDINBURGH. [*Received, April 24th,* 1952.]

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## 628. *Studies in Spectroscopy. Part I. Steric Effects in Some Substituted Butadienes and* cyclo*Butanes.*

By K. B. ALBERMAN, R. N. HASZELDINE, and F. B. KIPPING.

The ultra-violet absorption spectra of 1 : 1 : 4 : 4-tetraphenylbutadiene and of the compounds (I) where R = Ph, *p*-$C_6H_4Me$, *p*-$C_6H_4Pr^i$, and *p*-$C_6H_4Bu^t$ reveal appreciable steric effects in the molecules. Deductions are made concerning the configurations of the compounds.

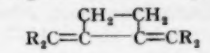
THE introduction of methyl groups into butadiene causes only small bathochromic shifts of about 50 Å for each methyl group, with only slight variation in intensity (Table 1). Introduction of a phenyl group at position 1 in butadiene, on the other hand, results

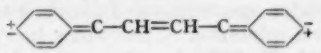
TABLE 1.

| | $\lambda_{max.}$ | $\varepsilon_{max.}$ | | $\lambda_{max.}$ | $\varepsilon_{max.}$ |
|---|---|---|---|---|---|
| Butadiene [1] ..... | 2170 | 20,900 | 2 : 3-Dimethylbutadiene [3] | 2260 | 21,400 |
| 1-Methylbutadiene [2] ..... | 2235 | 25,500 | 1 : 4-Dimethylbutadiene [2] | 2270 | 25,500 |
| Isoprene [1] ..... | 2200 | 23,900 | | | |

The solvent was hexane in all cases. [1] Smakula, *Angew. Chem.*, 1934, **47**, 657. [2] Booker, Evans, and Gillam, *J.*, 1940, 1453. [3] Scheibe, *Ber.*, 1926, **59**, 1333.

in a large bathochromic shift with increase in the intensity of absorption relative to butadiene and appearance of multiple maxima (Fig. 1 and Table 2, p. 3287); in *trans-trans*-1 : 4-diphenylbutadiene the main absorption band has increased still further in intensity (with loss of fine structure), and moved to longer wave-length. The discrepancies in the values in the literature are probably due to isomerisation of *trans-trans*-1 : 4-diphenylbutadiene to the *trans-cis*- and *cis-cis*-forms on irradiation (Sandoval and Zechmeister, *J. Amer. Chem. Soc.*, 1947, **69**, 553; Pinkard, Wille, and Zechmeister, *ibid.*, 1948, **70**, 1938; Hirshberg, Bergmann, and Bergmann, *ibid.*, 1950, **72**, 5150).

(I) 



(II)

Since the bathochromic shifts in the above compounds are due to increased conjugation, it might be expected that the introduction of more phenyl groups into the 1- and the 4-position of butadiene would lead to a further appreciable displacement of absorption maxima to the red, with increase in intensity. It has now been found, however, that the long-wave maximum of 1 : 1 : 4 : 4-tetraphenylbutadiene shows loss of fine structure, is of

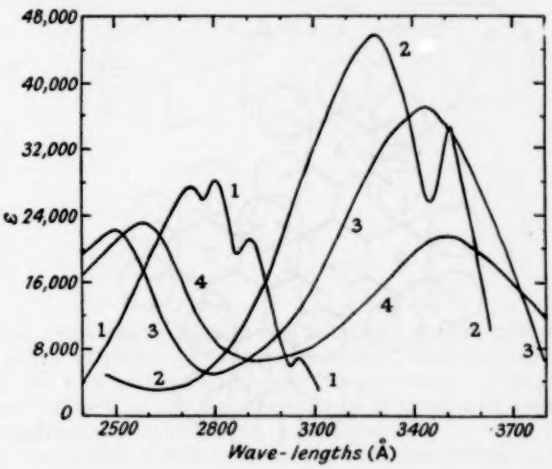



FIG. 1.

1, 1-*Phenylbutadiene*.
2, trans-trans-1 : 4-*Diphenylbutadiene*.
3, 1 : 1 : 4 : 4-*Tetraphenylbutadiene*.
4, 1 : 2-*Bisdiphenylmethylenecyclobutane*.

lower intensity, and is displaced to the red by only 150 Å relative to the corresponding band of 1 : 4-diphenylbutadiene (Fig. 1 and Table 2). The bathochromic effect is that which might be expected from the introduction into 1 : 4-diphenylbutadiene of two weakly chromophoric groups, and the spectrum thus indicates that the introduction of the two further phenyl groups has had little effect on the resonance in the molecule.

This may be explained on the basis of interaction between adjacent phenyl groups in 1 : 1 : 4 : 4-tetraphenylbutadiene as is revealed by a scale diagram (Fig. 2). Appreciable interference between the *ortho*-hydrogen atoms of the phenyl groups *A* with *B* and *C* with *D*, with some interference involving the hydrogen atoms attached to $C_{(2)}$ and $C_{(3)}$ of the butadiene system with those of rings *B* and *C*, is apparent when the four phenyl groups are coplanar. The molecule can assume a less strained configuration if rings *B* and *C* are twisted out of the plane of the rest of the system, *i.e.*, out of the plane of the paper. Since these rings cannot contribute to resonance structures of type (II) in which near coplanarity is essential, the ultra-violet spectrum of 1 : 1 : 4 : 4-tetraphenylbutadiene is presumably composed of the 1 : 4-diphenylbutadiene spectrum (displaced to the red by 150 Å) plus the weak absorption of the two independently absorbing out-of-plane phenyl groups ($\lambda$ *ca.* 2500 Å, $\varepsilon$ *ca.* 2 × 250). The decrease in intensity on passing from the 1 : 4- to the 1 : 1 : 4 : 4-substituted butadiene system indicates that complete coplanarity of rings *A* and *D* and the butadiene system cannot be realised even if rings *B* and *C* are at right angles to the plane of the paper. There can obviously be no isomerisation on irradi-

ation of 1 : 1 : 4 : 4-tetraphenylbutadiene similar to that reported for 1 : 4-diphenylbutadiene.

The short-wave-length maximum of 1 : 1 : 4 : 4-tetraphenylbutadiene at 2490 Å is comparable to the butadiene maximum displaced to the red by 320 Å by the introduction of four groups. The short-wave-length maximum of 1 : 4-diphenylbutadiene [unrecorded by Hausser, Kuhn, and Smakula, and by Blout and Eager (refs. in Table 2)] should thus be at *ca.* 2330 Å, and similarly 1 : 1 : 4-triphenylbutadiene should have a spectrum similar to that of 1 : 4-diphenylbutadiene with maxima at *ca.* 2410 and 3360 Å (ε *ca.* 21,000 and 40,000).

The ultra-violet spectrum of 1 : 1 : 4 : 4-tetraphenylbutadiene thus reveals another example of steric inhibition of resonance in systems containing the $-CH=CPh_2$ group. Jones (*ibid.*, 1943, **65**, 1820) has shown that 1 : 1-diphenylethylene has a spectrum similar to that of styrene and that tetraphenylethylene is spectroscopically similar to *trans*-stilbene. The introduction of a phenyl group into styrene to give 1 : 1-diphenylethylene caused a bathochromic shift approximately half that brought about in the long-wave-length band by the introduction of two phenyl groups into 1 : 4-diphenylbutadiene.

Steric inhibition of resonance has also been established for 1 : 2-disubstituted *cyclo*-

FIG. 2.

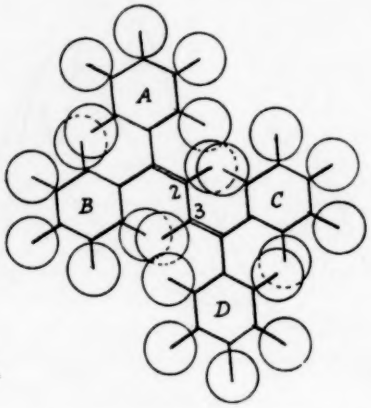
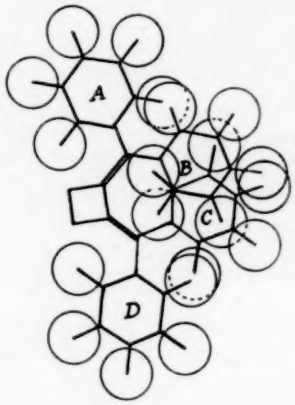



FIG. 3.




butane derivatives of type (I) where R is phenyl, *p*-tolyl, *p-iso*propylphenyl, or *p-tert.*-butylphenyl. These compounds may be regarded as butadiene in which $C_{(2)}$ and $C_{(3)}$ form part of the *cyclo*butane ring, and when R = Ph the tetraphenyl compound is clearly closely related to 1 : 1 : 4 : 4-tetraphenylbutadiene. Its spectrum (Fig. 1 and Table 2) shows that the band at shorter wave-length (2580 Å, ε 23,000) has the same intensity as that for tetraphenylbutadiene; the bathochromic shift of 90 Å can be attributed to the introduction of two alkyl groups into butadiene (regarding the *cyclo*butane ring as spectroscopically equivalent to two methyl groups). The long-wave-length band (3510 Å, ε 21,400) has undergone a similar expected bathochromic shift of 80 Å relative to tetraphenylbutadiene, but there is a considerable decrease in the intensity of absorption compared with di- and tetra-phenylbutadienes. This can be attributed to additional steric inhibition of resonance caused by introduction of the *cyclo*butane ring. A scale diagram (Fig. 3) shows the marked overlap between rings *A* and *B*, *C* and *D*, and *B* and *C*, if all the phenyl groups are coplanar.

The rotation of rings *B* and *C* through 90° about the bond joining them to the butadiene system still gives interference (Fig. 4; cf. 1 : 1 : 4 : 4-tetraphenylbutadiene where a similar rotation gives a strain-free configuration). The steric interaction in the *para*-positions of rings *B* and *C* of the *cyclo*butane compound could be relieved if these rings were forced apart by increase in bond angles (*e.g.*, if angles *x* and *x'* increased by about 8°, or if angles *y* and *y'* were also increased) and/or by change in bond length.

If the compound (I; R = Ph) had a configuration similar to that shown by Fig. 4, the substitution of groups in the *para*-position of rings *B* and *C* would increase considerably the steric effect. Furthermore, if the groups were sufficiently large the strain would be relieved only if none of the benzene rings were coplanar with the butadiene system, and a marked change in absorption spectrum and in the fluorescence exhibited by this type of compound should be observed. Introduction of alkyl groups into the *para*-position showed spectroscopically that the rings *B* and *C* cannot be at 90° to the plane of the *cyclo*-butane ring. The spectra of the compounds in question (Table 2) show bathochromic shifts and slight increases in intensity of absorption relative to the compound where R = Ph,

FIG. 4.

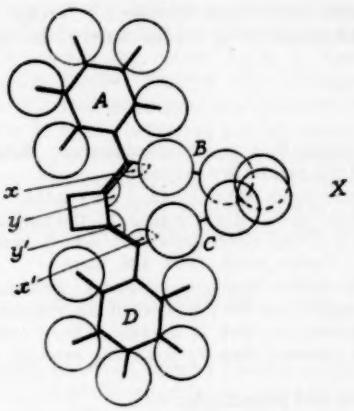
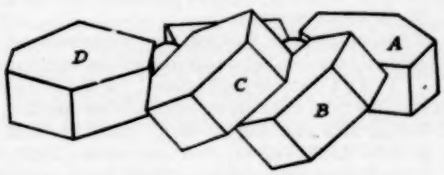



FIG. 5.




which can be explained only by the extra conjugation in the butadiene system due to groups in the *para*-positions such as Bu$^t$ contributing fully to resonance; that is to say, the butadiene system and two of the 1 : 4-substituted phenyl groups remain virtually coplanar. The maxima would have shifted to shorter wave-length if there had been appreciable non-planarity of the butadiene system involving rings *A* and *D*.

An alternative configuration for compounds of this type must therefore be sought and models show that the near coplanarity indicated by the absorption spectra can be maintained if rings *B* and *C* are parallel and at an angle to the rest of the system, as indicated in Fig. 5 by the edge-on view in the plane of the paper from *X* in Fig. 4.

It will, however, be noted that the intensities of the long-wave-length maxima of the *cyclo*butane derivatives are less than the corresponding maxima for tetraphenylbutadiene.

TABLE 2

| | First max. | | Second max. | | Fluorescence |
|---|---|---|---|---|---|
| 1-Phenylbutadiene [1] | 2710 | 28,000 | 2230 | 12,000 | Unrecorded |
| | 2800 | 28,300 | 2330 | 8,500 | |
| | 2900 | 27,000 | | | |
| | 3050 | 6,000 | | | |
| 1 : 4-Diphenylbutadiene [2] | 3280 | 41,000 | Unrecorded | | Deep blue |
| | 3500 | 35,000 | | | |
| 1 : 4-Diphenylbutadiene [3] | 3280 | 52,700 | Unrecorded | | |
| | 3450 | 34,000 | | | |
| Tetraphenylbutadiene | 3430 | 37,250 | 2490 | 22,800 | Deep blue |
| (I; R = Ph) | 3510 | 21,400 | 2580 | 23,000 | Intense green |
| (I; R = $C_6H_4Me$) | 3580 | 23,000 | 2610 | 28,400 | Yellow-green |
| (I; R = *p*-$C_6H_4Pr^i$) | 3630 | 23,400 | 2620 | 30,000 | Intense green |
| (I; R = *p*-$C_6H_4Bu^t$) | 3650 | 27,200 | 2630 | 32,200 | Intense yellow |

The solvent was ethanol in all cases. [1] Braude, Jones, and Stern, *J.*, 1947, 1087; Smakula, *loc. cit.*; Grummitt and Christopher, *J. Amer. Chem. Soc.*, 1949, **71**, 4157. [2] Hausser, Kuhn, and Smakula, *Z. physikal. Chem.*, 1935, *B*, **29**, 384. [3] Blout and Eager, *J. Amer. Chem. Soc.*, 1945, **67**, 1315.

A slight distortion of the butadiene system involving rings *A* and *D* of the *cyclo*butane compounds from the planar configuration giving maximum resonance interaction must therefore be involved even when the configuration shown by Fig. 5 is adopted. Models show that if rings *A* and *D* are twisted slightly out of the plane of the butadiene system, rings *B* and *C* can then be so disposed that their *p*-positions are not opposite one another and hence space is provided for the large substituent in, for example, the *tert.*-butyl compound. It is clear that a molecule represented by Fig. 5 should exhibit optical activity and experiments to demonstrate this are in hand.

Attempts to prepare *cyclo*butane derivatives in which R = 9-anthryl, and in which the butadiene type of resonance would be expected to be destroyed still further, failed; this is possibly because of steric factors involved in their formation.

The interatomic distances used in the diagrams are taken from Pauling's "Nature of the Chemical Bond," p. 220, and the angles of the *cyclo*butane ring are assumed to be 90°.

## Experimental

M.p.s are corrected.

*Apparatus.*—The spectra were measured on a Beckmann D.U. spectrophotometer; determinations were carried out in duplicate and the mean of the results is reported.

1 : 1 : 4 : 4-*Tetraphenylbutane*-1 : 4-*diol.*—This was prepared from diethyl succinate. It crystallises from chloroform in colourless needles, m. p. 206—207°. Dehydration by the method of Wittig and von Lupin (*Ber.*, 1928, **61**, 1627) gave 1 : 1 : 4 : 4-tetraphenylbuta-1 : 3-diene, m. p. 207—208° (Valeur, *Bull. Soc. chim.*, 1903, **29**, 683; *Compt. rend.*, 1903, **136**, 694).

cyclo*Butane Derivatives.*—These were prepared from diethyl *trans-cyclo*butane-1 : 2-dicarboxylate by treatment with the appropriate Grignard reagent and dehydration of the resulting carbinols (Alberman and Kipping, *J.*, 1951, 781). It is interesting that in the case of the *p*-tolyl compound the hydrocarbon, and not the carbinol, was isolated directly from the product of interaction of the ester and the Grignard reagent.

1 : 2-*Bisdiphenylmethylene*cyclo*butane.* (See Alberman and Kipping, *loc. cit.*)

1 : 2-*Bisdi*-p-*tolylmethylene*cyclo*butane.* The ester (2 g.) in ether was added to a Grignard solution from *p*-bromotoluene (8·6 g.) and magnesium (1·2 g.). After decomposition with ice and ammonium chloride in the usual way and steam-distillation, the residue (a yellow glass) crystallised from alcohol as a fluorescent microcrystalline powder. Recrystallisation from alcohol–benzene gave fine yellow needles of the *diene*, m. p. 214° (3 g.) (Found: C, 92·3; H, 7·7. $C_{34}H_{32}$ requires C, 92·7; H, 7·3%).

1 : 2-*Bisdi*-p-iso*propylphenylmethylene*cyclo*butane.* The carbinol from the ester (1 g.), *p*-bromocumene (5 g.) and magnesium (0·6 g.) formed colourless prisms from alcohol, m. p. 182—183° (decomp.). Dehydration yielded the *diolefin* as jagged plates (from alcohol–benzene), m. p. 148—149° (Found: C, 91·1; H, 8·5. $C_{42}H_{48}$ requires C, 91·3; H, 8·7%).

1 : 2-*Bisdi*-p-tert.-*butylphenylmethylene*cyclo*butane.* The *carbinol* from the ester (1 g.), *p*-bromo-*tert.*-butylbenzene (5·3 g.) (Marvel, *J. Amer. Chem. Soc.*, 1939, **61**, 2771) and magnesium (0·6 g.) crystallised from alcohol–benzene as colourless needles, m. p. 252° (2·3 g.) (Found: C, 85·5; H, 9·4. $C_{46}H_{60}O_2$ requires C, 85·7; H, 9·3%). Dehydration yielded the *diolefin* as yellowish blades (from benzene), m. p. 283—284° (Found: C, 91·0; H, 9·4. $C_{46}H_{56}$ requires C, 90·8; H, 9·2%).

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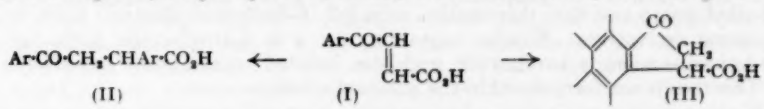
University Chemical Laboratory, Cambridge. [*Received, April 29th*, 1952.]

## 629. *The Preparation and Isomerisation of β-Aroylacrylic Acids.*

By G. Baddeley, G. Holt, and S. M. Makar.

Experimental procedure for the preparation of β-aroylacrylic acids by interaction of maleic anhydride and an aromatic hydrocarbon through the agency of aluminium chloride is discussed. Several of these acids have been isomerised to 3-ketoindane-1-carboxylic acids; ring closure of β-2-alkylaroylacrylic acids is sometimes accompanied by intramolecular migration of the 2-alkyl group. Decarboxylation of these ketoindanecarboxylic acids affords a valuable method of preparing indan-1-one derivatives.

Maleic anhydride reacts with aromatic hydrocarbons at room temperature in the presence of aluminium chloride (2 mols. as $AlCl_3$), to provide β-aroylacrylic acids (I); these have the *trans*-configuration (Lutz, *J. Amer. Chem. Soc.*, 1930, **52**, 3405; 1933, **55**, 1168, 1593; *J. Org. Chem.*, 1941, **6**, 77). Other reactions can and frequently do interfere, but they are avoided when practical procedure is based on the following considerations: (i) β-Aroylacrylic acids, behaving like αβ-unsaturated ketones, combine with aromatic hydrocarbons in the presence of hydrogen chloride and excess of aluminium chloride to provide β-aroyl-α-arylpropionic acids (II) (Pummerer and Buchta, *Ber.*, 1936, **69**, 1005). (ii) Even when excess of aromatic hydrocarbon is avoided, excess of aluminium chloride is detrimental as it affords less pure β-aroylacrylic acids and, in some instances, isomeric products; for

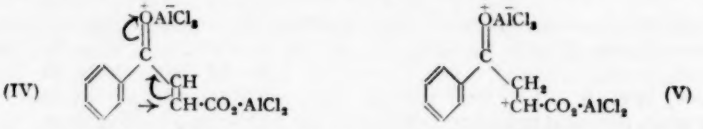



example, when durene, maleic anhydride, and aluminium chloride in the molecular ratio 1 : 1 : 4 are kept at room temperature for two days, 3-keto-4 : 5 : 6 : 7-tetramethylindane-1-carboxylic acid (X) is obtained in 85% yield (see Experimental section) by way of the intermediate β-2 : 3 : 5 : 6-tetramethylbenzoylacrylic acid (IX). The acid (IX), in methylene chloride, is not affected by excess of aluminium chloride if hydrogen chloride is swept from the solution by a stream of dry nitrogen, but (*a*) when hydrogen chloride is not removed, and especially (*b*) when the solution is saturated with dry hydrogen chloride, it affords the above indanone derivative acid in yields of (*a*) 20% and (*b*) 90% after one day. None of these complications arises if excess of aluminium halide, *i.e.*, more than 2 mols. per mol. of maleic anhydride, is avoided. β-Aroylacrylic acids are best prepared, therefore, by combining maleic anhydride with aluminium chloride (2 mols.) in methylene or ethylene chloride, decanting the solution from any undissolved aluminium chloride, and adding the aromatic hydrocarbon (1 mol.); yields are excellent. The success of this method rests on the high solubility of the complex $(CH{\cdot}CO)_2O,2AlCl_3$ and the low solubility of aluminium chloride in methylene or ethylene chloride; they enable the complex to be readily formed and easily freed from excess of aluminium halide. Fieser and Fieser (*J. Amer. Chem. Soc.*, 1935, **57**, 1679) obtained improved yields of β-aroylacrylic acids by employing trichloroethylene in place of carbon disulphide or nitrobenzene as solvent; their procedure, in which powdered aluminium chloride is added to a solution of aromatic hydrocarbon and maleic anhydride, exposes the reaction mixture to the action of the solid chloride and, therefore, cannot be recommended. Similar considerations have been applied in this laboratory to the preparation of β-aroylpropionic acids by interaction of succinic anhydride, aluminium halide, and aromatic hydrocarbons; this work will be reported later.

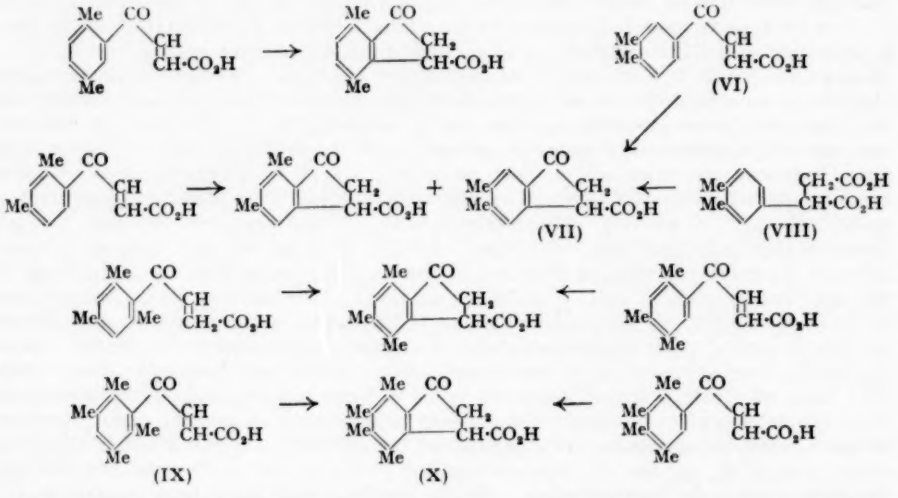
The yellow β-aroylacrylic acids obtained by interaction of maleic anhydride with toluene, *o*-, *m*-, and *p*-xylene, mesitylene, *pseudo*cumene, prehnitene, and durene severally afford colourless, isomeric 3-ketoindane-1-carboxylic acids (III) which do not decolorise cold aqueous permanganate or bromine in carbon tetrachloride. For example, β-3 : 4-dimethylbenzoylacrylic acid (VI) affords an isomeric acid which provides 5 : 6-dimethylindan-1-one on decarboxylation and is identical with the product of interaction of 3 : 4-dimethylphenylsuccinic acid (VIII) and sulphuric acid; it is, therefore, 3-keto-5 : 6-

dimethylindane-1-carboxylic acid (VII); Speight, Stevenson, and Thorpe (*J.*, 1924, 2190) showed that 3-ketoindane-1-carboxylic acid is obtained from phenylsuccinic acid and sulphuric acid.

We are unaware of any previous reference to the conversion of β-aroylacrylic acids into 3-ketoindane-1-carboxylic acids; it resembles the conversion of aryl vinyl ketones into indanones by the action of sulphuric acid or of aluminium and hydrogen chlorides in boiling carbon disulphide (*e.g.*, Smith and Pritchard, *J. Amer. Chem. Soc.*, 1940, **62**, 771) and is the intramolecular counterpart of (I ⟶ II). The electronic displacements can be represented as in (IV), but the dependence of the reaction on the presence of hydrogen chloride indicates that ring closure is preceded by addition of a proton to form a carbonium ion (V). As




expected, ease of ring closure increases with the number of alkyl groups attached to the benzene ring: whereas β-benzoylacrylic acid does not cyclise and the 4-methyl derivative does so to a small extent, higher homologues provide ketoindanecarboxylic acids in good yield. Recently we reported (*J.*, 1952, 2415) that ring closure of *o*-2-alkylaroylbenzoic acids through the agency of aluminium chloride is sometimes *preceded* by intramolecular migration of the 2-alkyl group and that this enables even *o*-2 : 6-dialkylaroylbenzoic acids to afford anthraquinone derivatives. Similar migrations occur in β-aroylacrylic acids but in no instance has an isomeric β-aroylacrylic acid been isolated; ring closure has always intervened. Our results are formulated in the annexed scheme.




Isomerisation of aryl ketones is a consequence of steric interaction between an acyl group and a bulky *ortho*-substituent as this partly inhibits mesomeric release of electrons from the aromatic ring to the acyl group and thereby makes the former more susceptible to attack by a proton. This does not occur in derivatives of indan-1-one because the five-membered ring ensures (i) comparatively little steric interaction between carbonyl group and a bulky substituent in the 7-position and (ii) coplanarity of the benzene ring and the carbonyl group (*J.*, 1944, 232; *Nature*, 1939, **144**, 444); therefore, migration of an alkyl group may precede or accompany the cyclisation of a β-aroylacrylic acid but cannot occur once the 3-ketoindane-1-carboxylic acid is formed.

All the above 3-ketoindane-1-carboxylic acids are readily decarboxylated by copper chromite in quinoline at 160—190° and afford the corresponding indanones in 65—70% yield; in consequence, the interaction of maleic anhydride and an aromatic hydrocarbon provides a valuable means of preparing derivatives of indan-1-one.

Interaction of maleic anhydride with quinol in the presence of excess of aluminium chloride follows a different course and affords naphthazarin (Zahn and Ochwat, *Annalen*, 1928, **462**, 72); in order to explain this remarkable difference, we are investigating the action of aluminium chloride on β-*o*-hydroxyaroylacrylic acids.

## Experimental

*β-Aroylacrylic Acids.*—Powdered maleic anhydride was added to a suspension of finely powdered aluminium chloride (2·5 mols.) in methylene or ethylene chloride, and the mixture agitated for 0·5 hour at room temperature. The solution was decanted from undissolved aluminium chloride, and an aromatic hydrocarbon (1 mol.) was added. After 0·5 hour, the reaction mixture was decomposed with dilute hydrochloric acid, solvent was removed by distillation, and the solid product recrystallised from benzene–light petroleum (1 : 1) or aqueous acetic acid. The following β-aroylacrylic acids were prepared in this way (yields in parentheses):

β-Benzoyl- (85%), m. p. 96—97° (Rice, *J. Amer. Chem. Soc.*, 1923, **45**, 222); β-*p*-methylbenzoyl- (70%), m. p. 137—138° (von Pechmann, *loc. cit.*); β-2 : 5-dimethylbenzoyl- (89%), m. p. 89—90° (Papa, Schwenk, and Villani, *J. Amer. Chem. Soc.*, 1948, **70**, 3356); β-2 : 4-dimethylbenzoyl- (91%), m. p. 117—118° (Kozniewski and Marchlewski, *Chem. Zentr.*, 1906, II, 1189); β-3 : 4-*dimethylbenzoyl*- (76%), m. p. 122—123° (Found: C, 70·6; H, 5·9. $C_{12}H_{12}O_3$ requires C, 70·6; H, 5·9%); β-2 : 4 : 6-trimethylbenzoyl- (83%), m. p. 140—141° (*idem, ibid.*); β-2 : 4 : 5-trimethylbenzoyl- (80%), m. p. 148—149° (*idem, ibid.*); β-2 : 3 : 5 : 6-*tetramethylbenzoyl*- (83%), m. p. 182—183° (Found: C, 72·2; H, 6·8. $C_{14}H_{16}O_3$ requires C, 72·4; H, 6·9%); and β-2 : 3 : 4 : 5-*tetramethylbenzoyl*- (76%), m. p. 122—123° (Found: C, 72·5; H, 6·9%).

*Interaction with Aluminium Chloride.*—This chloride (10 g.) and sodium chloride (1·5 g.) (both per g. of organic acid) were powdered together and heated by an oil-bath to 140°. The homogeneous melt was continuously stirred and brought to a selected temperature, and the organic acid gradually added. After a selected time the mixture was cooled and decomposed by ice and dilute hydrochloric acid, and the organic material separated and recrystallised from water.

(*a*) β-2 : 5-Dimethylbenzoylacrylic acid (10 g.), after 1 hour at 135°, provided 3-*keto*-4 : 7-*dimethylindane*-1-*carboxylic acid* (8·7 g.), m. p. 160—161° (Found: C, 70·4; H, 6·0%; equiv., 203. $C_{12}H_{12}O_3$ requires C, 70·6; H, 5·9%; equiv., 204). It formed a 2 : 4-dinitrophenylhydrazone, did not decolorise cold permanganate or bromine in carbon tetrachloride, and was oxidised by nitric acid (*d* 1·1) at 180° to benzene-1 : 2 : 3 : 4-tetracarboxylic acid, m. p. and mixed m. p. 244—246° (decomp.) (tetramethyl ester, m. p. and mixed m. p. 130—131°) (Read and Purves, *J. Amer. Chem. Soc.*, 1952, **74**, 117; Smith and Carlson, *ibid.*, 1939, **61**, 228; Fieser and Peters, *ibid.*, 1932, **54**, 4347). The isomerisation can be effected by aluminium chloride (3 mols.), but the initial acid is recovered when the chloride (2 mols.) is used. Sulphuric acid (75%) at 100° caused partial decomposition.

(*b*) β-2 : 4-Dimethylbenzoylacrylic acid (2 g.) and sulphuric acid (85%) gave 3-*keto*-4 : 6-*dimethylindane*-1-*carboxylic acid*, m. p. 157—158° (Found: C, 70·5; H, 6·0%; equiv., 204), in poor yield; it was oxidised by nitric acid to benzene-1 : 2 : 3 : 5-tetracarboxylic acid, m. p. and mixed m. p. 250—252° (tetramethyl ester, m. p. and mixed m. p. 107—109°) (Read and Purves, *loc. cit.*; Smith and Byrkit, *ibid.*, 1933, **55**, 4308; Nürsten and Peters, *J.*, 1950, 733). The acrylic acid (5 g.) with aluminium chloride at 135° during 1 hour gave a product (4 g.) from which 3-keto-4 : 6- (3·3 g.) and 3-*keto*-5 : 6-*dimethylindane*-1-*carboxylic acid* (0·2 g.), m. p. and mixed m. p. 172—174° (Found: C, 70·1; H, 6·0%), were obtained by repeated recrystallisation from water. The latter was also obtained by the action of sulphuric acid (85%) at 100°, and of aluminium chloride at 100° on β-3 : 4-dimethylbenzoylacrylic acid, and in yields of *ca.* 15% by the action of sulphuric acid, phosphoric oxide in boiling toluene, or aluminium chloride–sodium chloride on 3 : 4-*dimethylphenylsuccinic acid*. This acid was obtained as colourless needles, m. p. 198—199° (Found: C, 65·3; H, 6·5. $C_{12}H_{14}O_4$ requires C, 64·9; H, 6·3%), from 3 : 4-dimethylbenzaldehyde as described by Lapworth and Baker (*Org. Synth.*, 1927, **7**, 20; 1928, **8**, 88) for the preparation of phenylsuccinic acid from benzaldehyde. α-*Cyano*-β-3 : 4-*dimethylphenylacrylic acid*, which was obtained as intermediate, crystallised from ethanol in pale yellow plates, m. p. 205—206° (Found: C, 71·5; H, 5·2. $C_{12}H_{11}O_2N$ requires C, 71·6;

H, 5·5%). 3-Keto-5 : 6-dimethylindane-1-carboxylic acid was oxidised by nitric acid (*d* 1·1) to benzene-1 : 2 : 4 : 5-tetracarboxylic acid, m. p. and mixed m. p. 284—287° (tetramethyl ester, m. p. and mixed m. p. 140—142°) (Read and Purves, *loc. cit.*; Fieser and Campbell, *J. Amer. Chem. Soc.*, 1938, **60**, 2635; Feist, *Ber.*, 1911, **44**, 135). 3-Keto-4 : 6-dimethylindane-1-carboxylic acid did not provide the 5 : 6-isomer when heated with aluminium chloride.

(*c*) β-2 : 4 : 6-Trimethylbenzoylacrylic acid (4·7 g.), after 3 hours at 135° or 1 hour at 150°, gave 3-*keto*-4 : 6 : 7-*trimethylindane*-1-*carboxylic acid* (3·9 g.), m. p. 189—190° (Found: C, 71·3; H, 6·5%; equiv., 220. $C_{13}H_{14}O_3$ requires C, 71·6; H, 6·4%; equiv., 218). It is a saturated acid, forms a 2 : 4-dinitrophenylhydrazone, is oxidised by nitric acid (*d* 1·1) at 220° to benzenepentacarboxylic acid, m. p. and mixed m. p. 225—230° (decomp.) (pentamethyl ester, m. p. and mixed m. p. 145—147°) (Ruzicka and Rudolph, *Helv. Chim. Acta*, 1927, **10**, 919; Jacobsen, Stromberg, and Peterson, *J. Amer. Chem. Soc.*, 1949, **71**, 1384; Fleischer and Retze, *Ber.*, 1923, **56**, 228), and is identical (m. p. and mixed m. p.) with the product of interaction of β-2 : 4 : 5-trimethylbenzoylacrylic acid with sulphuric acid (85%) or aluminium chloride at 100°.

(*d*) β-2 : 4 : 5-Trimethylbenzoylacrylic acid, after 1 hour at 100° or at 150°, provided only 3-keto-4 : 6 : 7-trimethylindane-1-carboxylic acid.

(*e*) β-2 : 3 : 5 : 6-Tetramethylbenzoylacrylic acid (5 g.), after 1 hour at 105°, gave 3-*keto*-4 : 5 : 6 : 7-*tetramethylindane*-1-*carboxylic acid* (4·5 g.), m. p. 216—217° (from water) (Found: C, 72·2; H, 6·8. $C_{14}H_{16}O_3$ requires C, 72·4; H, 6·9%). It is identical (m. p. and mixed m. p.) with the product of isomerisation of β-2 : 3 : 4 : 5-tetramethylbenzoylacrylic acid after 1 hour at 110°, and affords mellitic acid, m. p. 286—288° (sealed tube) (hexamethyl ester, m. p. 187—188°) (Fieser and Fieser, "Organic Chemistry," D. C. Heath & Co., Boston, U.S.A., 1950, p. 705), by oxidation with nitric acid.

(*f*) β-Benzoylacrylic acid partly decomposed during unsuccessful attempts to isomerise it at 105° or 145° (1 hour); β-*p*-toluoylacrylic acid, after 2 hours at 120°, afforded only 5% of an isomeric saturated acid, m. p. 146—147° (Found: C, 69·7; H, 5·2. $C_{11}H_{10}O_3$ requires C, 69·5; H, 5·3%), which is probably 3-*keto*-6-*methylindane*-1-*carboxylic acid*.

*Decarboxylation of* 3-*Ketoindane*-1-*carboxylic Acids*.—The acid (2 g.) and copper chromite (0·5 g.) in quinoline (10 c.c.) were heated in an atmosphere of nitrogen; evolution of carbon dioxide commenced at 160—165° and was complete after 3—4 hours at 180—190°. After cooling, the mixture was diluted with ether (100 c.c.) and filtered, the quinoline extracted with dilute acid, the ether removed by evaporation, the residue distilled with steam, and the indanone separated and crystallised from acetic acid. The following derivatives of indan-1-one were prepared in this manner (yields in parentheses): 4 : 7-Dimethyl- (64%), needles, m. p. 78—79° (Plattner and Wyss, *Helv. Chim. Acta*, 1941, **24**, 483) (phenylhydrazone, needles, m. p. 131—132°, from ethanol) (Maureu, *Bull. Soc. chim.*, 1893, **9**, 568); 5 : 7-*dimethyl*- (65%), prisms, m. p. 75—76° (Found: C, 82·1; H, 7·4. $C_{11}H_{12}O$ requires C, 82·5; H, 7·5%); 5 : 6-*dimethyl*- (43%), plates, m. p. 83—84° (Found: C, 82·1; H, 7·6%); 4 : 5 : 7-trimethyl- (68%), pale yellow needles, m. p. 110—111° (oxime, plates, m. p. 227—228°, from ethanol) (Aitken, Badger, and Cook, *J.*, 1950, 333, give m. p.s 104—105° and 223—224° respectively); 4 : 5 : 6 : 7-tetramethyl- (71%), yellow plates, m. p. 152—153° (oxime, plates, m. p. 214—215°, from ethanol) (Aitken, Badger, and Cook, *loc. cit.*, give m. p.s 149—150° and 211—213° respectively).

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## 630. *The Heat of Formation of Disodium Methylphosphonate.*

By RAYMOND THOMPSON.

The heat of combustion of disodium methylphosphonate has been determined in a commercial bomb calorimeter, and a value of 226·6 ± 0·5 kcal./mole obtained. The equation for the bomb reaction has been established quantitatively to be $CH_3{\cdot}PO(ONa)_2(c) + 2O_2(g) = \frac{1}{2}Na_4P_2O_7(c) + CO_2(g) + \frac{3}{2}H_2O(l)$, from which the heat of formation of disodium methylphosphonate (c) is calculated to be 350 kcal. (1465 abs. k-joules)/mole.

EVALUATION of heats of formation from heats of combustion of organic compounds containing elements other than carbon, hydrogen, nitrogen, and oxygen is not readily accomplished owing to difficulties involved in ascertaining the precise stoicheiometry of the bomb

reaction. The products of combustion often include acids of the other elements (*e.g.*, sulphur and the halogens) in various states of hydration and oxidation. Uniformity of hydration is guaranteed only by the use of a rotating bomb such as that used by Sunner (*Svensk Kem. Tidskr.*, **1946**, **58**, 71); oxidation is dependent to some extent on the presence and action of nitric and other acids (*e.g.*, Huffman and Ellis, *J. Amer. Chem. Soc.*, **1935**, **57**, 41), and the fixation and subsequent analysis of a mixture of chemically similar acids can introduce considerable practical problems. There is no record of the combustion of organophosphorus compounds, and it was considered that, in view of the numerous oxy-acids of phosphorus and their various polymeric forms, the bomb reaction might in this case be even more complex. A difficulty encountered in the actual combustion of such compounds was the low volatility of the acids produced. For example, attempts to burn methylphosphonic acid were unsuccessful, for the acid produced damped combustion, and only by using an accelerator in proportion so large as to render the heat contribution due to the methylphosphonic acid relatively small (with consequent reduced accuracy) could the substance be induced to burn completely.

The experiments now described were therefore designed with a view to the complete elimination of liquid and gaseous phosphorus compounds as products of combustion. This has been achieved by burning disodium methylphosphonate, which was found (following J. K. Leary's qualitative observation) to be converted quantitatively into sodium pyrophosphate according to the equation

$$CH_3{\cdot}PO(ONa)_2(c) + 2O_2(g) = \tfrac{1}{4}Na_4P_2O_7(c) + CO_2(g) + \tfrac{3}{2}H_2O(l)$$

Sodium pyrophosphate remained within the crucible after combustion as a crystalline white solid. Under optimum conditions combustion was complete and no carbonaceous residue remained. The water formed condensed in droplets on the walls of the bomb; a trace of phosphate was detectable in this aqueous phase.

The heat of combustion was found to be 226·6 ± 0·5 kcal./mole (weighed in air) at 21°, and the value was assumed to be the same as 25°. By using the value of 760·8 kcal./mole for the heat of formation of sodium pyrophosphate (c) ("Selected Values of Chemical Thermodynamic Properties," Nat. Bur. Stand., 1950) and 94·05 and 68·32 for carbon dioxide (g) and water (l) respectively (Wagman, Kilpatrick, Taylor, Pitzer, and Rossini, *J. Res. Nat. Bur. Stand.*, **1945**, **34**, 143), the heat of formation of disodium methylphosphonate is seen to be 350 kcal. (1465 abs. k-joules)/mole.

That the solid remaining after combustion was sodium pyrophosphate was revealed by qualitative tests and verified by an *X*-ray powder photograph. The stoicheiometry of the bomb reaction was established as follows. The sodium salt burned was perfectly pure and anhydrous, and a phosphate determination on the combined solid and liquid reaction products gave P, 22·10% [Calc. for $CH_3{\cdot}PO(ONa)_2$: P, 22·13%]. The weight of sodium pyrophosphate remaining in the crucible in those experiments in which scattering did not occur (see below) corresponded with the above equation and the weight of disodium methylphosphonate taken. The following results are typical:

| | Calc. | Expt. 1 | Expt. 2 |
|---|---|---|---|
| Weight ratio $2CH_3{\cdot}PO(ONa)_2/Na_4P_2O_7$ ..... | 1·054 | 1·052 | 1·053 |

Fused sodium pyrophosphate is not readily hydrated and has a high vapour pressure in equilibrium with the hydrated salt (there are no lower hydrates than the decahydrate). In view of this and the facts that (*a*) water formed during combustion condenses preferentially on the cold walls of the bomb, (*b*) the vapour pressure of water within the bomb is small, and (*c*) the crucible was weighed immediately following the determination, it was expected that the degree of hydration would be slight. This was confirmed by a determination of the phosphorus content of the solid within the crucible (Found: P, 23·08. Calc. for $Na_4P_2O_7$: P, 23·29%). The close proximity of the above ratios to the theoretical is therefore due to the mutually compensating effects of this small degree of hydration and of slight volatilisation. A ratio of 1·056 could be obtained by withdrawing the crucible 2—3 minutes after firing.

It is thus established that, within the precision of the apparatus used, the heat evolved during the combustion of disodium methylphosphonate is entirely that due to the reaction indicated by the above equation.

## Experimental

*Preparation of Materials.*—Methylphosphonic acid was prepared by hydrolysis of methylphosphonyl dichloride, 65 g. of which were added, slowly and with constant stirring, to 50 ml. of water. The resultant liquid was then evaporated to dryness, a further 50 ml. of water added, and the process repeated. By this means all traces of hydrogen chloride were removed, and 40 g. of a wax-like solid remained on cooling.

The disodium salt was prepared by dissolving 24 g. of the acid in 50 ml. of dry ethanol and treating the solution with 23 g. of sodium dissolved in 400 ml. of ethanol. A white precipitate formed rapidly, but the mixture was refluxed for ½ hour to complete the reaction. The product was removed by filtration and recrystallised twice from 50% aqueous ethanol, then dried in a vacuum-desiccator. About 30 g. of the solvated product were obtained, purity rather than high yield being the primary consideration.

The salt was prepared for combustion by drying it successively in ovens at 80° (3 hours), 140° (16 hours) and 180° (4 hours). It was then compressed into firm pellets, which were transferred to a weighing bottle and further dried at 180° for 3 hours.

*Calorimetry.*—Heats of combustion were determined by using a Mahler–Cook bomb calorimeter and standard procedure. The water equivalent of the apparatus was assessed by 12 combustions of benzoic acid (B.D.H. Thermochemical Standard), the heat of combustion of which was taken to be 6·319 kcal./g. (vac.). Temperature measurements were made with a Beckmann thermometer which had been calibrated against a standard. The relative mean deviation of the water equivalent values was calculated to be 0·2%, a deviation high by comparison with modern standards but of adequate precision for present purposes. All combustions were performed under a pressure of 25 atm. and at 21°±1°, and all values corrected to 15°-calories. Washburn corrections were not applied.

The pellets of salt were weighed from the weighing bottle into a silica crucible of 2·5-cm. top diameter and 2 cm. high; this relatively deep crucible was necessary in order to prevent scattering of the contents during combustion. Ignition was effected by means of a 4 × 0·009 cm. platinum wire and a potential of 12 v in conjunction with a fuse (0·005 g.) of oven-dried Whatman No. 1 filter-paper; the heat of combustion of the paper was taken to be 3·99 kcal./g. (C. B. Knowles, Thesis, Manchester, 1951). It was found that complete combustion of the salt was not obtained if the sample exceeded 1·8 g.; the size and shape of the crucible were partly responsible for this critical weight, and a black core resulted if larger quantities were burned. About 1·6 g. were used in obtaining the combustion values quoted. The capacity of the bomb was 300 ml., and no water was introduced before combustion.

The heat of combustion of disodium methylphosphonate was taken as the mean of six determinations whose individual values were: 226·3, 226·7, 227·2, 226·4, 226·9, 225·9 kcal./mole.

On a number of occasions several small pieces of solid sodium pyrophosphate were found on the base of the bomb after combustion. It was seen from their sharp edges, and corresponding crevices in the pyrophosphate within the crucible, that this was due to uneven contraction during cooling and not to scattering at the time of combustion. Silica was known to be soluble in molten phosphate (Yost and Russell, "Systematic Inorganic Chemistry," Prentice Hall, 1944, p. 224) but the amount actually dissolved in the short time during which the sodium pyrophosphate was molten was only about 0·4 mg. It was not possible to assess alkalimetrically the amount of nitric acid produced during the combustion of disodium methylphosphonate because of the large excess of sodium pyrophosphate. The thermal contribution due to nitric acid formation was therefore considered to be the same as in the case of the combustion of benzoic acid (9 cal.), and the heats of combustion recorded have been calculated upon this assumption. Any error incurred thereby (and the results of spectrophotometric analysis suggest that nitric acid formation is the same) is not of significance in comparison with certain other errors attendant upon the present method of calorimetry, and particularly of temperature measurement.

*Analysis.*—The phosphorus content of the combustion products was determined as magnesium pyrophosphate following double precipitation. It must be emphasised that, contrary to general belief, pyrophosphate is not readily converted completely into orthophosphate by dissolution in water and boiling, even after the addition of hydrochloric acid. In the present work 25-ml. aliquots containing 0·25 g. of sodium pyrophosphate were refluxed for several hours

with 5 ml. of concentrated nitric acid, finally being diluted to 100 ml., and the determination completed in the normal manner.

Spectrophotometric estimation of nitrate present in the bomb after combustion was effected with a Unicam SP 500 spectrophotometer. The ultra-violet-absorbing substances present were pyrophosphate, nitrate, and dissolved silica, of which only the last was found to absorb at 370 mμ. The absorption due to silica at 302·5 mμ was readily calculated by application of data obtained from heating " AnalaR " sodium pyrophosphate in a silica crucible in air and measuring the absorption of the aqueous solution of the product. Knowing the weight of sodium pyrophosphate in the bomb contents and $\varepsilon_{302\cdot5}$ (found by separate experiments for the same concentration of $Na_4P_2O_7$ in water to be 0·07), and employing a value for sodium nitrate of $\varepsilon_{302\cdot5} = 7\cdot01$ (Addison, Gamlen, and Thompson, *J.*, 1952, 338), the amount of nitrate present was readily determined.

The author expresses his thanks to Professor H. V. A. Briscoe for granting facilities for the work, and to Dr. A. J. E. Welch, in whose laboratory the research was done, for co-operation and advice.

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## 631. *The Preparation and Magnetic Susceptibility of Clathrate Compounds of Nitric Oxide and of Oxygen.*

By D. F. EVANS and R. E. RICHARDS.

Methods have been devised for preparing clathrate compounds of quinol and oxygen, and of quinol and nitric oxide. The specific susceptibilities of the compounds have been measured, and the values of $\chi$ for the enclosed molecules calculated. For oxygen in the clathrate compound, $\chi = 108\cdot8 \times 10^{-6}$, which agrees within experimental error with measurements in the gas phase. For nitric oxide in its compound, $\chi = 51\cdot_6 \times 10^{-6}$. This value is higher than the recorded results for gaseous nitric oxide, but the difference may not be significant. It is pointed out that the magnetic properties of these compounds at very low temperatures might be of interest.

POWELL and his collaborators (*J.*, 1948, 815) described in detail the structures of a number of adducts formed by quinol and molecules or atoms of suitable dimensions, such as methanol, sulphur dioxide, and inert gases. In these so-called clathrate compounds, each trapped molecule is totally enclosed in a cage formed by the molecular arrangement of the β-quinol lattice. It was thought that the physical properties of molecules thus trapped in an environment different from that normally present in the pure material, might prove of interest; in particular, it seemed likely that the magnetic properties of clathrate compounds of paramagnetic substances would be easy to study. Of the common paramagnetic gases, oxygen and nitric oxide were chosen, as these are the only ones of suitable size which do not react with quinol.

### EXPERIMENTAL

*Preparations.*—(a) *The oxygen clathrate compound.* A hot concentrated solution of quinol in air-free ethyl alcohol in a Monel-metal pressure bomb was allowed to cool slowly during 24 hours under a pressure of 25—40 atm. of oxygen. The faintly yellow crystals obtained were filtered off and dried in a vacuum desiccator; they are perfectly stable under normal conditions at room temperature, but on exposure to strong sunlight gradually turn brown. When dissolved in water, the compound evolves a gas, and analysis by Dr. B. Lambert and Mr. A. B. Webster, by absorption in Fieser's solution, showed this to contain 97·6% of oxygen. The volume of gas obtained from a known weight of sample was measured in a nitrometer and corresponded in different samples to clathrate compounds in which 40—50% of the available spaces had been filled, *i.e.*, the compound is in the range $3C_6H_4(OH)_2,0\cdot4—0\cdot5O_2$. The densities of the samples were consistent with this interpretation.

(b) *The nitric oxide clathrate compound.* A warm saturated solution of quinol in ethyl

alcohol was boiled to remove dissolved air, and poured into a stainless-steel bomb. The bomb was evacuated with a rotary oil pump and quickly cooled in liquid oxygen. Nitric oxide, prepared by the method of *Inorg. Synth.*, **2**, p. 126, in an evacuated vessel, was passed through an evacuated train of traps containing sodium hydroxide solution to remove nitrogen peroxide, and sodium hydroxide pellets to remove water, and condensed into the bomb. The quantities of sodium nitrite and ferrous sulphate used in the preparation were chosen so that a final pressure of about 40 atm. would be obtained in the bomb at room temperature. After its valve had been closed, the bomb was removed from the liquid oxygen and finally immersed in hot water. It was shaken for some time to redissolve all the quinol and then allowed to cool slowly for about 24 hours. Excess of nitric oxide was removed by evacuating the bomb, and the crystals were filtered off from the mother-liquor. They were washed with a saturated solution of quinol in ethyl alcohol to remove small amounts of quinhydrone formed by oxidation of the quinol, and were then dried in a vacuum desiccator. The crystals evolved nitric oxide when dissolved in solvents. The samples were analysed by adding air-free water to a known weight in an evacuated vessel, and measuring the pressure developed by means of a mercury manometer. The purity of the nitric oxide was found by repeating this analysis, an alkaline solution of sodium sulphite being used in place of water to dissolve the clathrate compound. This reagent is a quantitative absorbent for nitric oxide (Divers, *J.*, 1899, **75**, 82). The samples contained amounts of nitric oxide which corresponded to about 50% of the available space. The purity of the nitric oxide evolved from one sample was found to be 98·5%. Crystals of this clathrate compound were also obtained by using water as a solvent, but these samples contained much smaller quantities of nitric oxide.

The nitric oxide compound forms crystals which are stable in dry air, but when finely powdered slowly turn violet.

*Magnetic Susceptibilities.*—The susceptibility measurements were made with a Gouy apparatus. The sample was contained in an egg-shell-thick glass tube, suspended by a fine copper wire from one arm of a Sartorius microbalance. The lower end of the specimen was centred in the magnetic field of an electromagnet which provides fields of about 8000 gauss. The current through the electromagnet was kept at a fixed value by manual adjustment of a small series resistance, a potentiometric arrangement being used to measure the current accurately. The magnet was usually operated near the flat part of its hysteresis curve.

The apparatus was calibrated by using air-free distilled water in the sample tube. It was assumed for water that the specific susceptibility, $\chi$, is $-0{\cdot}720 \times 10^{-6}$, and for air at room temperature, the volume susceptibility, $\kappa$ is $0{\cdot}029 \times 10^{-6}$. The reproducibility of the apparatus with water is about $\pm 0{\cdot}1\%$, as shown by the following data (by R. E. Richards and C. O. L. Juulman):

| Temp. | Press., mm. | $d$ | Force on $H_2O$, mg. | Temp. | Press., mm. | $d$ | Force on $H_2O$, mg. |
|---|---|---|---|---|---|---|---|
| | (Specimen No. 1) | | | | (Specimen No. 2) | | |
| 19·76° | 763·0 | 0·99825 | 22·108 | 19·60° | 739·3 | 0·99829 | 22·074 |
| 20·35 | 762·0 | 0·99813 | 22·110 | 19·00 | 744·4 | 0·99841 | 22·094 |
| 20·40 | 760·2 | 0·99812 | 22·105 | 20·20 | 743·9 | 0·99816 | 22·067 |
| 19·63 | 757·2 | 0·99828 | 22·113 | 20·00 | 742·9 | 0·99820 | 22·070 |

The systematic difference between the results for the two specimens is probably due to differences in their purity. Experiments carried out with organic liquids such as benzene showed no systematic differences of this kind for different fillings of the tube, since these substances are easier to purify and free completely from dissolved oxygen. (These results are to be published shortly.)

The solid samples were finely powdered and packed as evenly as possible into the Gouy tube, and in the case of the oxygen compound the uniformity of this packing is responsible for the largest error (about 1—2%). A small correction was applied for the air contained in between the particles of solid in the tube; this was calculated from the measured density of the packed sample and from the known density of the crystals.

*The oxygen compound.* The results obtained for the specific susceptibility ($\chi \times 10^6$) at 17° are given below:

| | | |
|---|---|---|
| Filling 1 ..... | 3·318, 3·322, 3·310 | Mean = 3·317 |
| Filling 2 ..... | 3·276 | |

Average value of $\chi$ at 17° = $+ 3{\cdot}29_7 \times 10^{-6}$.

The sample was analysed in the nitrometer after the susceptibility measurements, and found to have 39·0% of the available spaces filled. The purity of the oxygen evolved was 97·6%.

The specific susceptibility ($\times 10^6$) of quinol has been given as $-0{\cdot}586$ (Palacious and Foz, *Anal. Fís. Quím.*, 1935, **33**, 627), $-0{\cdot}604$ (French, *Trans. Faraday Soc.*, 1945, **41**, 676), and $-0{\cdot}588$ (Mikhail and Baddar, *J.*, 1944, 590). The values $0{\cdot}587$ and $0{\cdot}604 \times 10^{-6}$ lead to a specific susceptibility for the oxygen enclosed in the clathrate compound of 108·6 and $109{\cdot}0 \times 10^{-6}$, respectively. The experimental values of $\chi$ for gaseous oxygen at 17° range from $104{\cdot}5$ to $110{\cdot}0 \times 10^{-6}$ (Selwood, "Magnetochemistry," p. 117, Interscience Publ., New York). The theoretical value calculated by Van Vleck is $107{\cdot}7 \times 10^{-6}$ ("Electric and Magnetic Susceptibilities," O.U.P.). This shows that the susceptibility of the oxygen molecules in the clathrate compound is very little affected by their environment. Facilities for measuring the value of $\chi$ for the clathrate compound at very low temperatures are not available, but the value might well be of interest as the diamagnetic $O_4$ complex could not be formed under these conditions.

*The nitric oxide compound.* When the finely powdered nitric oxide compound was packed into the Gouy tube, rapid decomposition occurred, with eventual formation of quinhydrone and benzoquinone. This is probably due to small amounts of liberated nitric oxide which are oxidised by the air to nitrogen peroxide, which reacts with the quinol. More nitric oxide and also water are liberated from this reaction, and cannot escape from the tightly packed tube of powder, so that further decomposition of the clathrate compound ensues. Accordingly, susceptibility measurements were made immediately after packing the tube, and the sample was then quickly removed and analysed. In spite of these precautions, however, the accuracy of the measurements is considerably lower than in the case of the oxygen compound. The results are shown below.

| Sample no. | Spaces filled, % | Purity, % | $\chi_{\text{Clathrate}} \times 10_6$ | Temp. | $\chi_{NO} \times 10^6$ | $\chi_{NO} \times 10^6$, corr. to 17° |
|---|---|---|---|---|---|---|
| 1 | 51·1 | 98·5 (ass.) | $1{\cdot}62_6$ | 18° | 50·4 | 50·6 |
| 2 (*a*) | 49·1 | 98·5 | $1{\cdot}56_7$ | 17·6 | 51·0 | 51·1 |
| (*b*) | 48·2 | 98·5 | $1{\cdot}64_6$ | 17 | 53·8 | 53·8 |

Average value at 17° = $51{\cdot}_8 \times 10^{-6}$.

(In this table $\chi$ for quinol is assumed to be $-0{\cdot}604 \times 10^{-6}$. If it is taken as $-0{\cdot}587 \times 10^{-6}$, an average value of $\chi_{NO}$ at 17° of $51{\cdot}_4 \times 10^{-6}$ is obtained.)

The experimental values of $\chi \times 10^6$ for gaseous nitric oxide at 17° have been given as 47·7 (Burris and Hause, *J. Chem. Physics*, 1943, **11**, 442), 49·2 (Bauer, Weiss, and Pickard, *Compt. rend.*, 1918, **167**, 484), and 49·3 (Sone, *Sci. Rep. Tohoku Univ.*, 1922, **11**, 139). The value calculated by Van Vleck (*op. cit.*) is $48{\cdot}3 \times 10^{-6}$.

The specific susceptibility of the nitric oxide molecule in the clathrate compound appears to be higher than in the gas phase, but, owing to the difficulty of handling this compound, this difference may not be significant. More precise results could probably be obtained by using an evacuated Gouy tube.

The normal state of the nitric oxide molecule is a regular $^2\Pi$ doublet, the energy difference between the $^2\Pi_{\frac{1}{2}}$ and the $^2\Pi_{\frac{3}{2}}$ state being about 352 cal./mole. The effective Bohr magneton number of nitric oxide therefore varies with temperature, and at 17° is 1·83. At low temperatures this number should fall to zero, in accordance with equations given by Van Vleck (*op. cit.*, p. 269), but since this gas liquefies at 121° K, and forms diamagnetic $N_2O_2$, it has not been possible to verify this below 112·8° K. The nitric oxide clathrate compound would provide a method of extending the measurements to very low temperatures. It is possible that these two low-lying energy levels of nitric oxide might be perturbed by some kind of interaction between the nitric oxide molecules and the cage, and give rise to the disrepancy between the values of the susceptibility of the gas and that found for the clathrate compound.

We are grateful to Mr. H. M. Powell for the loan of a stainless-steel bomb, and Dr. L. E. Sutton for a Monel-metal bomb. One of us (D. F. E.) is indebted to the Department of Scientific and Industrial Research for a maintenance grant.

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## 632. *Surface Isomerisation. Part I. The Surface-catalysed Racemisation of 2-Phenylbutane.*

By A. G. Davies and G. A. H. Elton.

Optically active 2-phenylbutane recovered after adsorption on carbon has been found to have undergone partial racemisation, the degree of racemisation depending on the time and temperature of residence on the carbon. A tentative explanation of this phenomenon is given in terms of homolytic fission of the hydrocarbon on the surface of the adsorbent.

It has been postulated that one of the functions of a heterogeneous catalyst may in some cases be to disrupt reacting molecules into free radicals on adsorption, the distance between adsorption sites and the structure of the adsorbate molecule being among the factors which determine the position of fission in the adsorbed molecule (Waters, "The Chemistry of Free Radicals," 2nd Edn., Oxford Univ. Press, 1948, pp. 214—221; Melville, *Ann. Reports*, 1938, **35**, 71—77). In this paper we give further evidence for the occurrence of homolysis produced by surface adsorption, based on measurements of optical activity of a substance before and after adsorption on carbon surfaces.

Carbon was chosen as a suitable adsorbent because of its high specific surface area, which was necessary for the convenient adsorption of a sufficient quantity of material for use in measurements of optical activity.

In choosing a suitable compound for study, attention was given to the following points: (1) In order to minimise the possibility of heterolytic (ionic) fission occurring on adsorption, use of a hydrocarbon appeared preferable. (2) The hydrocarbon chosen must be capable of resolution, a fact which drastically limits the available choice. (3) The hydrocarbon must be of the type which is likely to undergo homolysis at the asymmetric centre. Homolytic fission normally occurs readily at a tertiary hydrogen atom; choice was thus directed to compounds of the type $CR^1R^2R^3H$. (4) In order that the radical shall be fairly readily formed at moderate temperatures, it is preferable that one of the groups $R^1$, $R^2$, or $R^3$ should be a phenyl group, so that the radical formed is resonance stabilised. Since 2-phenylbutane fulfils all these requirements and has been previously resolved (Harrison, Kenyon, and Shepherd, *J.*, 1926, 658), it was chosen for these studies. That 2-phenylbutane can undergo homolysis at the asymmetric centre of the molecule is indicated by its autoxidation to form 2-phenyl-2-butyl hydroperoxide (Hawkins, *J.*, 1949, 2076), and by the fact that this reaction is catalysed by ultra-violet light (Ivanov, Savinova, and Zhakhovskaya, *Doklady Akad. Nauk. S.S.S.R.*, 1948, **59**, 905). Similar, more comprehensive evidence exists for the free-radical reactions of 2-phenylpropane (Hock and Lang, *Ber.*, 1944, **77**, 257; Kharasch, McBay, and Urrey, *J. Org. Chem.*, 1945, **10**, 401; Farmer and Moore, *J.*, 1951, 131).

Two widely differing specimens of carbon were used in the experiments: (*A*) "Norit" and (*B*) a granular animal charcoal. Adsorption was brought about by passing a stream of nitrogen saturated with the hydrocarbon vapour over the carbon, or by admitting the vapour to the thoroughly evacuated adsorbent. The carbon carrying the adsorbed material was then heated to *ca.* 200° for varying lengths of time, in order to determine the effect of different residence conditions. In the early experiments attempts were made to desorb the hydrocarbon by passing over it a stream of pure nitrogen while keeping the carbon at a temperature well above the normal boiling point of the hydrocarbon (172°). This was found to remove only a small fraction of the total adsorbate, and the hydrocarbon recovered by this method, which was probably only capillary-adsorbed, had undiminished rotation. The carbon carrying the remainder of the hydrocarbon, which was probably held by chemisorption and van der Waals forces, was a dry powder with no trace of the characteristic smell of the hydrocarbon. This strongly adsorbed material could be partly recovered from the carbon by heating it under a high vacuum and high temperature, or completely by extracting it with hot benzene, and the hydrocarbon thus obtained had a diminished

rotation, the degree of loss of activity depending on its treatment while on the adsorbent. The results of typical experiments are summarised in the table.

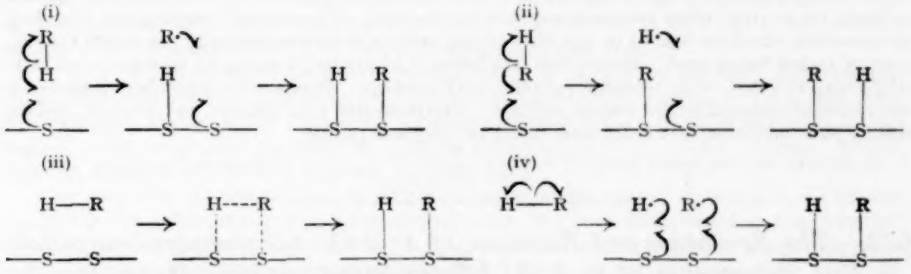
| Expt. | Specimen of carbon | Heating: time, hrs. | Heating: temp., etc. | Activity of hydrocarbon, $a_D$ ($l = 0{\cdot}5$): initial | Activity of hydrocarbon, $a_D$ ($l = 0{\cdot}5$): final | Loss of activity, % |
|---|---|---|---|---|---|---|
| 1 | *B* | — | — | −1·00° | −0·92° | 8 |
| 2 | *A* | 1 | 100°/25 mm. | +1·50 | +1·41 | 6 |
| 3 | *B* | 1 | 190 | −1·02 | −0·86 | 16 |
| 4 | *B* | 1 | 200 | +1·47 | +1·28 | 13 |
| 5 (i) | *B* | *ca.* 2 | 200/$10^{-4}$ mm. | +0·38 | +0·31 * | 18 |
| (ii) | | *ca.* 5 | | | +0·20 † | 47 |

* Pumped off at $10^{-4}$ mm.
† Extracted with benzene after further desorption by pumping became impossible.

A control experiment in which the hydrocarbon was heated to 200° for 1 hour in a sealed tube produced no racemisation; therefore it seems safe to conclude that the racemisation observed in our experiments is the result of the adsorption–desorption process. Chemisorption or strong van der Waals adsorption appears necessary for the racemisation, since the loosely held hydrocarbon, which was probably capillary-condensed, was recovered with unchanged rotation.

*Discussion.*—This racemisation of the hydrocarbon might have taken place (*a*) during the process of adsorption, (*b*) while adsorbed on the carbon surface, or (*c*) during the desorption process.

(a) *The adsorption process.* At least four possible mechanisms of chemisorption may be considered, *viz.*, (i)—(iv). Mechanism (i) would involve formation of a planar free radical with consequent racemisation; (ii) might involve inversion of configuration in the group R; (iii) is unlikely to produce racemisation; and (iv) would involve complete racemisation even in the absence of a surface, and our control experiment has shown that this does not occur.




(b) *Reaction in the adsorbed layer.* Two types of mechanism are usually postulated to account for reaction on a catalyst surface (Eley, *Quart. Reviews*, 1949, **3**, 209). (i) Reaction between adjacent molecules or radicals chemisorbed on the surface. This might lead to the formation of R–H, or R–R and H–H. If R–H is produced, desorption would probably occur and racemisation might then be brought about by re-adsorption by any of the above mechanisms. Appreciable formation of dehydro-dimer (R–R), and corresponding amounts of hydrogen, is excluded since almost quantitative recovery of the original hydrocarbon is made experimentally. (ii) The reaction between molecules or radicals in the chemisorbed layer with molecules in the van der Waals adsorbed layer:

This would probably involve extensive racemisation.

(c) *The desorption process.* Any of the four postulated adsorption mechanisms may function in reverse, although the reverse of reactions (i) and (iv) seems rather improbable. The fact that, as shown in the table, the time and temperature of residence on the carbon

markedly affect the degree of racemisation of the hydrocarbon indicates that at least a large portion of this racemisation must occur during stage (*b*). Of the two possible mechanisms given above, (*b*, ii) is the one normally recognised as being effective at moderate temperatures such as those employed in our experiments.

The results of experiments (1) and (2) indicate that only a small degree of racemisation is obtained if the hydrocarbon is desorbed immediately after adsorption, or after only a small amount of heating on the surface. This would suggest that racemisation during processes (*a*) and (*c*), if occurring at all, is of relatively small importance.

In view of the above results, it would appear to be of value to study other cases in which surface homolysis may lead to isomerisation. Studies of the surface-catalysed geometrical isomerisation of maleic and fumaric acids are in progress in this laboratory, and it is hoped to extend the investigation to other substances, *e.g.*, *cis*- and *trans*-decahydronaphthalene.

## Experimental

*Materials.*—Carbon *A*, a commercial specimen of finely-divided "Norit," was shaken with dilute sulphuric acid, washed, shaken with dilute sodium hydroxide, thoroughly washed, and dried at 120°. It was then refluxed for a prolonged period with absolute alcohol, then with pure benzene, and dried at 120° under vacuum. Carbon *B*, a granular animal charcoal, was similarly purified.

2-Phenylbutane, prepared by Hawkins's method (*J.*, 1949, 2076), was resolved by Harrison, Kenyon, and Shepherd's method (*loc. cit.*). The various specimens were distilled directly before use (b. p. 171—172°).

*Optical Activities.*—These were measured on the pure liquid at room temperature in a 5-cm. tube, sodium-*D* light being used.

*Adsorption and Desorption.*—Two methods of adsorption were used: (i) A slow stream of pure dry nitrogen was passed through the hydrocarbon (*ca.* 1·5 g.) kept at about 60°, and the resulting vapour led up a column packed with the adsorbent (*ca.* 30 g.) kept at a slightly higher temperature to prevent mechanical condensation. (ii) About 60 g. of the carbon were evacuated at $10^{-4}$ mm. and 200° for a prolonged period, after which the outgassed hydrocarbon was allowed to distil on to it at room temperature from a side arm. The carbon carrying the adsorbed hydrocarbon was then heated *in situ* for varying periods, a thermostatically controlled electric heating jacket being used. Desorption was brought about by pumping at $10^{-4}$ mm. and 200° into a trap at −80°, or by repeated refluxing with benzene. It was never possible to remove all the adsorbed material by the former method. The recovered hydrocarbon was distilled, and its boiling point and refractive index were taken as criteria of purity.

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## 633. *The Resolution and Reactions of* 1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthol. A New Preparation of* 1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthyl Hydroperoxide.*

By A. G. Davies and A. M. White.

1 : 2 : 3 : 4-Tetrahydro-1-naphthol has been resolved and its optically active acetate and hydrogen phthalate described. The tendency for these compounds to react by alkyl–oxygen fission is demonstrated by (*a*) the dependence of the rotatory power of the alcohol, obtained by hydrolysis of the hydrogen phthalate, upon the nucleophilic power of the hydrolytic reagent, (*b*) the formation of a sulphone from the alcohol and the hydrogen phthalate by reaction with sodium toluene-*p*-sulphinate in formic acid solution, and (*c*) the racemisation and formation of olefin by the (+)-hydrogen phthalate in acid solution. A new reaction, proceeding by an alkyl–oxygen fission mechanism, is the preparation of 1 : 2 : 3 : 4-tetrahydro-1-naphthyl hydroperoxide by reaction of the above hydrogen phthalate with 90% hydrogen peroxide.

For an investigation of 1 : 2 : 3 : 4-tetrahydro-1-naphthyl hydroperoxide, we wished to obtain the corresponding alcohol in an optically active state and to determine the ease with which it, and its derivatives, would undergo alkyl–oxygen fission.

1 : 2 : 3 : 4-Tetrahydronaphthalene was autoxidised to the hydroperoxide, and reduction of this with sodium sulphite gave 1 : 2 : 3 : 4-tetrahydro-1-naphthol as a viscous oil which crystallised after being seeded with a specimen of the solid (+)-alcohol. The alcohol was esterified by reaction with phthalic anhydride in the presence of pyridine, and the hydrogen phthalate resolved * by crystallisation of its quinidine salt from acetone solution until the melting point and specific rotatory power of the hydrogen phthalate attained a maximum value. The maximum rotatory power of the alcohol was established by hydrolysis of this ester with sodium ethoxide in 96% ethyl alcohol; from this alcohol the hydrogen phthalate was regenerated with undiminished rotatory power, confirming that no racemisation had occurred during the hydrolysis. It may thus be assumed that hydrolysis under these conditions proceeds by an acyl–oxygen fission mechanism, and that alcohol and hydrogen phthalate of similar sign of rotation have corresponding configurations. The optically active acetate was prepared by esterification of the optically pure alcohol.

The three lines of evidence presented below suggest that 1 : 2 : 3 : 4-tetrahydro-1-naphthol and its derivatives exhibit a tendency to react by alkyl–oxygen fission. Firstly, hydrolysis of the optically pure hydrogen phthalate with aqueous sodium carbonate gave the alcohol with retained configuration but only 11% of its maximum rotatory power. This is presumably due to the incursion of a unimolecular alkyl–oxygen fission mechanism (Day and Ingold, *Trans. Faraday Soc.*, 1941, **37**, 686), as has been established in the case of analogous arylmethanols (Kenyon *et al.*, *J.*, 1942, 556, 605; 1946, 797, 803, 807; 1951, 376, 380, 382, 385, 386). Secondly, both the alcohol and its hydrogen phthalate readily yield 1 : 2 : 3 : 4-tetrahydro-1-naphthyl *p*-tolyl sulphone by reaction with sodium toluene-*p*-sulphinate in formic acid solution. This reaction necessitates alkyl–oxygen fission in the alcohol and ester and has been used as diagnostic of a tendency to reaction by that mechanism (Kenyon *et al.*, *loc. cit.*). No formation of sulphone was observed in acetic acid solution with added sulphuric acid. Thirdly, the hydrogen phthalate undergoes slow racemisation in acetic acid solution containing a trace of sulphuric acid; after 8 hours at 60° the rotatory power of the hydrogen phthalate had fallen to 60% of the initial value. It was optically and chemically stable in acetic acid solution at 60°, but in formic acid solution, after 2 hours at 47°, 1 : 2-dihydronaphthalene was isolated.

1 : 2 : 3 : 4-Tetrahydro-1-naphthyl derivatives thus appear to undergo alkyl–oxygen fission reactions somewhat more readily than the corresponding 1-phenylethyl compounds. For example, hydrolysis of 1-phenylethyl hydrogen phthalate with aqueous sodium carbonate yields an alcohol retaining 90% of the maximum activity (Balfe, Downer, Evans, Kenyon, Poplett, Searle, and Tárnoky, *J.*, 1946, 797).

The methods of preparation of alkyl hydroperoxides are of two types. The first method is the autoxidation of a hydrocarbon; this has been interpreted as a free-radical reaction, and occurs most readily at the α-position to an ethylenic double bond or benzene ring (Farmer, Bloomfield, Sundralingham, and Sutton, *Trans. Faraday Soc.*, 1942, **38**, 348). 1 : 2 : 3 : 4-Tetrahydro-1-naphthyl hydroperoxide has previously been prepared only by this method (Hock and Susemihl, *Ber.*, 1933, **66**, 61; this paper). The second general method involves the use of hydrogen peroxide; all the published reactions of this type can be interpreted as involving nucleophilic attack of the $HO{\cdot}O^-$ ion or HO·OH molecule on a carbon atom according to the general equation :

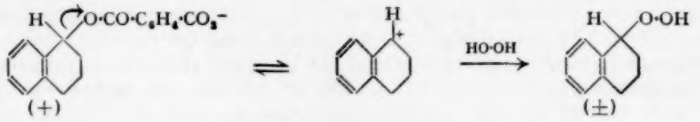
$$HO{\cdot}O^- + R\overset{\curvearrowright}{-}X \longrightarrow HO{\cdot}OR + X^-$$

(Davies and White, *Nature*, in the press).

It has now been established that 1 : 2 : 3 : 4-tetrahydro-1-naphthyl hydroperoxide, identical with the product obtained by autoxidation, is formed by reaction of 1 : 2 : 3 : 4-tetrahydro-1-naphthyl hydrogen phthalate with 90% hydrogen peroxide. In view of the established tendency towards alkyl–oxygen fission in the ester, and the fact that the inactive hydroperoxide is obtained from the optically active ester, it would seem that this

* The reference to a previous resolution of this alcohol which is given in Heilbron's " Dictionary of Organic Compounds," Vol. III, p. 698, is apparently an error and refers to the partial resolution of 1 : 2 : 3 : 4-tetrahydro-2-naphthol by Pickard and Littlebury (*J.*, 1906, **89**, 1254).

reaction may be included in the second of the above two classifications, and proceeds by a unimolecular alkyl–oxygen fission mechanism, as follows:




## Experimental

**1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthyl Hydroperoxide*.**—1 : 2 : 3 : 4-Tetrahydronaphthalene (1500 g.) was autoxidised at 75—78° for 50—60 hours (*Org. Synth.*, 1940, **20**, 94), and the product (102 g.) recovered by Robertson and Waters's method (*J.*, 1948, 1578). Recrystallisation from light petroleum gave the hydroperoxide (52 g.) as large rhombs, m. p. 55°.

**1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthol*.**—The hydroperoxide (50 g.) was reduced by shaking it with an aqueous solution of sodium sulphite (125 g.) for 5 hours (Hock and Lang, *Ber.*, 1942, **75**, 713). 1 : 2 : 3 : 4-Tetrahydro-1-naphthol (37 g., 83%), b. p. 136°/19 mm., was obtained; this solidified to a waxy crystalline solid, m. p. 29—30°, after being seeded with a solid specimen of the (+)-alcohol (Mentzer and Billet, *Bull. Soc. chim.*, 1948, 835, record m. p. 33°). The phenylurethane had m. p. 122—123°.

**1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthyl Hydrogen Phthalate*.**—The alcohol (30 g.) in dry benzene (15 c.c.) was added to phthalic anhydride (30 g.) in dry redistilled pyridine (24 g., 1·5 mols.); after 5 hours at 60° and 12 hours at room temperature the mixture, diluted with an equal volume of acetone, was poured on 1·5 l. of crushed ice and acidified (Congo red) with 5N-hydrochloric acid. After 2 days the **1 : 2 : 3 : 4-*tetrahydro*-1-*naphthyl hydrogen phthalate*** solidified (53 g., 83%). Recrystallisation from carbon disulphide–light petroleum gave clusters of needles, m. p. 112° (Found: C, 72·7; H, 5·1%; equiv., 296. $C_{18}H_{16}O_4$ requires C, 73·0; H, 5·4%; equiv., 296). A similar procedure, but with the omission of benzene, gave only a 45—50% yield of a less pure product.

**(+)-1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthyl Hydrogen Phthalate*.**—The (±)-hydrogen phthalate (50 g.) in acetone (50 c.c.) was added to a hot solution of anhydrous quinidine (55 g.) in acetone (600 c.c.). On cooling, large crystals of the solvated quinidine salt separated (87 g.); seven recrystallisations from acetone gave the optically pure salt, $[\alpha]_D^{20}$ +63·2° (*l*, 2; *c*, 2·1 in chloroform). This (+)-quinidine salt (31 g.), as a slurry in acetone (150 c.c.), was rendered homogeneous by the addition of 5N-hydrochloric acid, which solution was filtered on to crushed ice (750 c.c.) and confirmed to be acid (Congo-red). After 4 days the precipitated (+)-hydrogen phthalate solidified; it was purified by dissolution in ether and 3 extractions with 3N-hydrochloric acid. Recrystallisation of the crude material (12 g., 81%) from carbon disulphide gave short rods, m. p. 122°, $[\alpha]_D^{18}$ +11·1° (*l*, 2; *c*, 4·1 in chloroform). Decomposition of the quinidine salt from the mother liquors gave, after 3 recrystallisations from carbon disulphide, the (−)-hydrogen phthalate, m. p. 122°, $[\alpha]_D^{18}$ −10·5° (*l*, 2; *c*, 6·8 in chloroform).

***Hydrolysis of* (+)-1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthyl Hydrogen Phthalate*.**—(a) *With sodium ethoxide in ethyl alcohol.* A mixture of the (+)-hydrogen phthalate (3 g.), $[\alpha]_D^{18}$ +11·1° (*l*, 2; *c*, 4·1 in chloroform), in 96% ethyl alcohol (40 c.c.), and sodium (0·5 g., 2·5 mols.) in the same solvent (10 c.c.) was heated under reflux for 1 hour, the mixture poured into water, and the resulting alcohol recovered by extraction with ether. After distillation the (+)-1 : 2 : 3 : 4-tetrahydro-1-naphthol (1·0 g., 67%) solidified to a white micro-crystalline solid, b. p. 92°/1·8 mm., m. p. 39—40°, $[\alpha]_D^{17}$ +32·65° (*l*, 2; *c*, 2·5 in chloroform), $[\alpha]_D^{17}$ +8·54° (*l*, 2; *c*, 2·0 in carbon disulphide). This alcohol was converted back into the (+)-hydrogen phthalate with undiminished rotation.

(b) *With aqueous sodium carbonate.* A solution of the (+)-hydrogen phthalate (2 g.), $[\alpha]_D^{18}$ +11·1° (*l*, 2; *c*, 4·1 in chloroform) in aqueous sodium carbonate (0·9 g., 2·5 mols.; in 25 c.c. of water) was heated on the steam-bath for 15 minutes and the mixture then distilled in a current of steam. After extraction with ether, the distillate yielded the (+)-alcohol (0·7 g., 70%), b.p. 92°/2 mm., m. p. 29° $[\alpha]_D^{19}$ + 3·6° (*l*, 2; *c*, 3·7 in chloroform). This alcohol was converted into the hydrogen phthalate which then had no detectable activity (*l*, 2; *c*, 4·4 in chloroform).

**(−)-1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthyl Acetate*.**—A mixture of the (+)-alcohol (0·5 g.), $[\alpha]_D^{17}$ +32·65° (*l*, 2; *c*, 2·5 in chloroform), acetic anhydride (0·38 g.), and pyridine (0·3 g.), after 6 hours at 60°, was poured into water (100 c.c.) containing hydrochloric acid (10 c.c.; 3N-solu-

tion). The washed and dried ethereal extract yielded (−)-1 : 2 : 3 : 4-*tetrahydro*-1-*naphthyl acetate* (0·5 g., 78%), b. p. 112—113°/2·5 mm., $n_D^{20}$ 1·5292, $d_4^{25}$ 1·0816; $\alpha_D^{21}$ −58·1° (homogeneous; *l*, 0·5); $[\alpha]_D^{21}$ −107·4° (homogeneous; *l*, 0·5) (Found: C, 76·0; H, 7·0. $C_{12}H_{14}O_2$ requires C, 75·8; H, 6·8%). The (±)-acetate obtained by the same method from the (±)-alcohol had $n_D^{20}$ 1·5307. Criegee (*Annalen*, 1930, **481**, 263) reported b. p. 105—110°/2 mm.

1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthyl* p-*Tolyl Sulphone.*—(a) *From the alcohol.* A mixture of the (±)-alcohol (0·50 g.) and sodium toluene-*p*-sulphinate (0·80 g.) in 98% formic acid (20 c.c.) was kept overnight at room temperature, and was then poured on crushed ice (100 c.c.); the precipitated *sulphone* (0·62 g., 62%) was recrystallised from methyl alcohol forming well-formed rhombs, m. p. 131° (Found: S, 11·3. $C_{17}H_{18}O_2S$ requires S, 11·2%). The use of glacial acetic acid (20 c.c.) and concentrated sulphuric acid (2 drops) as solvent instead of formic acid, after 8 hours at 60°, led to the recovery of the unchanged alcohol (96%). At 100° a similar reaction mixture gave a brown non-distillable material which was not further investigated.

(b) *From the hydrogen phthalate.* The hydrogen phthalate (0·50 g.), dissolved in a little acetone, was added to a solution of sodium toluene-*p*-sulphinate (0·50 g.) in 98% formic acid (15 c.c.). After 15 minutes at 100° and 48 hours at 20° characteristic crystals of the sulphone were precipitated. More was obtained (total, 0·36 g., 75%) by pouring the reaction mixture on crushed ice. Recrystallisation from methyl alcohol gave the sulphone, m. p. 131°.

*Racemisation of the* (−)-*Hydrogen Phthalate in Acid Solution.*—From a similar reaction mixture in glacial acetic acid in the presence of sulphuric acid, no sulphone was isolated, but the (−)-hydrogen phthalate was recovered partially racemised: the (−)-hydrogen phthalate (1·0 g.), m. p. 119°, $[\alpha]_D^{21}$ −8·5° (*l*, 2; *c*, 8·3 in chloroform) and sodium toluene-*p*-sulphinate (1·0 g.) were dissolved in glacial acetic acid (20 c.c.) containing 2 drops of concentrated sulphuric acid. After 8 hours at 60°, the hydrogen phthalate (0·76 g.) was recovered; it had m. p. 115—117°, $[\alpha]_D^{20}$ −5·2° (*l*, 2; *c*, 6·3 in chloroform). At 100° a brown non-distillable material was again obtained.

*Reaction Between* (−)-*Hydrogen Phthalate and* 98% *Formic Acid.*—A solution of the (−)-hydrogen phthalate (2·5 g.), $[\alpha]_D^{20}$ −4·8° (*l*, 2; *c*, 2·8 in chloroform), in 98% formic acid (20 c.c.), after 2 hours at 47°, was poured into water (50 c.c.) and extracted with ether. From the dried ethereal extract, 1 : 2-dihydronaphthalene was obtained (0·30 g.), b. p. 56—58°/0·9 mm.; $n_D^{20}$ 1·5820 (Found: C, 92·1; H, 7·8. Calc. for $C_{10}H_{10}$: C, 92·3; H, 7·7%). A non-distillable residue (0·46 g.) was obtained, which could not be solidified. The involatile residues, obtained in this and the preceding two experiments, may be polymerised dihydronaphthalenene.

From a similar reaction in glacial acetic acid solution, after 8 hours at 60°, the unchanged (−)-hydrogen phthalate was recovered with undiminished rotation.

*Reaction of* (+)-1 : 2 : 3 : 4-*Tetrahydro*-1-*naphthyl Hydrogen Phthalate with Hydrogen Peroxide.*—The (+)-hydrogen phthalate (1·1 g.), $[\alpha]_D^{19}$ +11·1° (*l*, 2; *c*, 4·1 in chloroform), was added to 90% hydrogen peroxide (10 c.c.) containing sodium hydrogen carbonate (1·4 g.) at 0°. An oil soon separated on the surface of the initially homogeneous solution. After 2 days at 0°, water was added (30 c.c.); the ethereal extract yielded, after recrystallisation from light petroleum, 1 : 2 : 3 : 4-tetrahydro-1-naphthyl hydroperoxide (0·35 g.), m. p. 54—56° undepressed by mixture with the hydroperoxide obtained by autoxidation of tetralin. No optical activity could be detected in the product (*l*, 0·5; *c*, 18·6 in chloroform).

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BATTERSEA POLYTECHNIC, LONDON, S.W.11. [*Received, May 22nd,* 1952.]

## 634. *The Decomposition of Sulphuric Acid in Town's Gas Flames.*

By R. F. Littlejohn and G. Whittingham.

Dew-point measurements of the combustion products have been used to determine the degree of decomposition of sulphuric acid injected into pre-mixed town's gas flames. The initial concentration of acid in the gases was in the range 0·006—0·12% (by vol.), and 94—98% is decomposed in Bunsen-type flames. Studies on separated flames show that the decomposition occurs almost entirely in the inner cone. The mechanism of decomposition is discussed with particular reference to the reaction between sulphur trioxide and atomic hydrogen.

There is evidence that the behaviour of sulphuric acid and sulphur trioxide, arising from the combustion of sulphur-bearing fuels, is of importance in relation, not only to the corrosive nature of the combustion products, but also to formation of carbon in flames of burning hydrocarbons. Thus it has been shown (Whittingham, *Nature*, 1945, **156**, 207) that the addition of 4% of sulphur dioxide to a town's gas diffusion flame inhibited carbon formation. This effect was confirmed by Gaydon and Whittingham (*Proc. Roy. Soc.*, 1947, *A*, **189**, 313), and Parker and Wolfhard (*Fuel*, 1950, **29**, 235), and the latter authors also showed that sulphur trioxide behaved in the same way as sulphur dioxide in diffusion flames. Whittingham (*Trans. Faraday Soc.*, 1948, **44**, 141) observed that the production of tar in the slow combustion of hexane–air–sulphur dioxide mixtures coincided with the appearance of sulphuric acid in the combustion products, and the significance of production of sulphur trioxide in relation both to deposition of carbon and to corrosive wear in engines has been discussed by several workers (Cloud and Blackwood, *Soc. Automotive Engrs.*, 1943, **51**, 408; Broeze and Wilson, *Inst. Mech. Engrs.*, 1948—49, 128).

A knowledge of the stability of sulphuric acid at high temperatures is essential to the fuller understanding of its effect on combusion processes. Information exists on its heterogeneous dissociation over a limited temperature range (*e.g.*, Mellor, "Treatise on Theoretical and Inorganic Chemistry," Vol. 10, p. 396) but the rate of decomposition in flame processes has not previously been investigated. The subject has been examined in the present work, with particular reference to pre-mixed town's gas flames.

### Experimental

Controlled amounts of sulphuric acid were sprayed into various flames by a spray burner of the type described by Stockton (*J. Sci. Instr.*, 1949, **26**, 157) and the sulphuric acid dew-point of the products of combustion was measured. In the first series of experiments Bunsen-type flames were used, and in a second series the apparatus was modified to act as a Smithells separator so that the lower and the upper cone could be separately examined.

The two assemblies used are illustrated in Fig. 1. In the first, the supplies of town's gas and primary and secondary air fed to the burner were measured by capillary flowmeters, and the amount of acid entering the flame was varied by altering the concentration of acid in the solution in the reservoir. A side-arm attached to the base of the Pyrex chimney enabled sulphur dioxide to be introduced from a syphon, so that its behaviour could be compared with that of sulphuric acid. In the experiments with separated flames, the mixture fed to the burner was deficient in air, and the products of the primary reaction burned to completion as a diffusion flame at the top of the chimney. The amount of air diffusing into this flame was not measured but it was possible to calculate it from the water-vapour content of the interconal gases and the final products. The water-vapour content was obtained from measurement of the water dew-point and an Orsat apparatus was used to analyse the interconal gases for carbon dioxide, carbon monoxide, and oxygen.

The dew-point technique used to determine the quantities of sulphur trioxide and water in the combustion products was the same as that previously described (Flint, *J. Inst. Fuel*, 1948, **21**, 248). The essential feature of the apparatus is an air-cooled glass element, in which are mounted two platinum–platinum–rhodium thermocouples for measurement of the temperature and electrical conductivity of the surface. The element is placed in contact with the hot gases and cooled by a stream of air directed against its under side. Condensation is indicated by a

rapid increase in conductivity. Data (Taylor, *J Inst. Fuel*, 1942, **16**, 25; Dooley and Whittingham, *Trans. Faraday Soc.*, 1946, **42**, 354) on gases having a wide range of sulphuric acid and water contents were used to obtain the acid content from the measured dew-point.

## Results

*Bunsen-type Flames.*—Town's gas and primary and secondary air were supplied at the rates of 4·5, 11·25, and 34·25 l. min.$^{-1}$ respectively. The town's gas had the average composition by volume: $CO_2$ 3·6, $O_2$ 0·4, $C_nH_m$ 4·6, CO 15·0, $CH_4$ 23·7, $H_2$ 42·4, and $N_2$ 10·3%. If $C_nH_m$ is assumed to be $C_4H_8$, the calculated composition of the combustion products was $CO_2$ 5·6, $H_2O$ 10·0, $O_2$ 10·0, $N_2$ 74·4%, giving a theoretical water dew-point of 46°, whereas the measured dew-point was 50°. The difference was attributed to the presence of traces of residual sulphur compounds in the gas, which oxidise to sulphur trioxide and thereby produce a slightly higher dew-point.

The quantities of acid induced into the flame, for various solutions in the reservoir, are shown

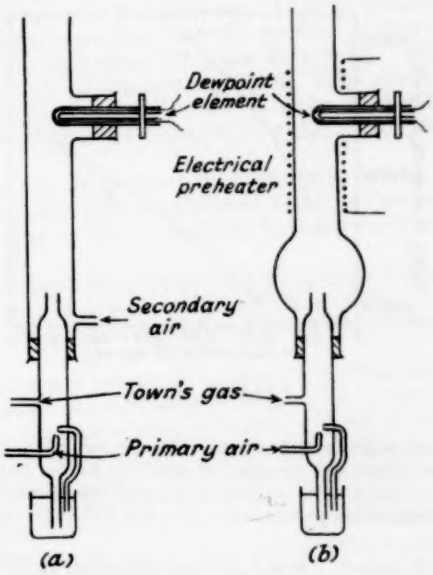



Fig. 1. *Apparatus used for (a) Bunsen-type flames, (b) separated flames.*

in Table 1. The reduction in the rate of spraying, as the concentration of acid in solution increased, was due to the increasing density and viscosity of the solution.

When acid was sprayed into the flame, carbon was deposited on the walls of the mouth of the burner over a length of about 0·5 cm., and with the 20% and 40% solution a shower of carbon particles appeared in the flame. The sulphuric acid concentration of the combustion products

Table 1.

| $H_2SO_4$ in solution (% by vol.) | Rate of spraying (c.c./hr.) | Amount of acid introduced (% by vol. of air–gas mixture) |
|---|---|---|
| 2·5 | 16·5 | 0·0060 |
| 5·0 | 15·0 | 0·0110 |
| 10·0 | 12·0 | 0·0176 |
| 20·0 | 11·0 | 0·0322 |
| 40·0 | 6·0 | 0·0351 |

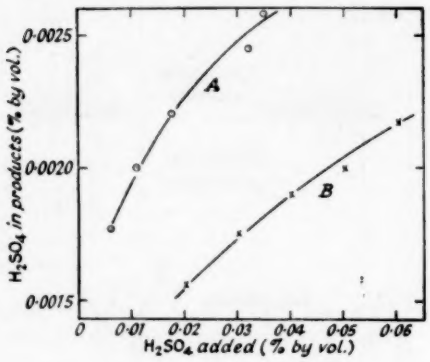
is plotted against the ingoing sulphuric acid in Fig. 2. The lower curve shows the amount of acid produced for a range of sulphur dioxide additions.

*Separated Flames.*—Town's gas and air were supplied to the lower cone at the rates of 3·35

and 8·7 l. min.$^{-1}$ respectively and the range of acid additions was as before. Analysis of the combustion products from this flame, when uncontaminated, gave the average values $CO_2$ 7·6, CO 9·7, $O_2$ 0·0, $H_2O$ 11·5, and $N_2$ 71·2% (by difference). Because of the cooling of the products of the lower flame as they traversed the chimney and the large amounts of dilution air induced in the upper diffusion flame, it was difficult to maintain a stable flame. Some degree of stability was achieved by electrical preheating of the interconal gases by about 200°.

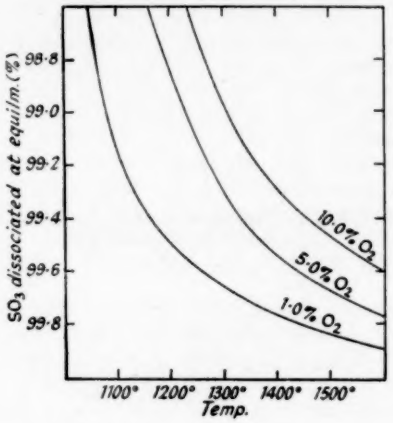
The measured water dew-point of the final combustion products was 41°, corresponding to 7·0% of water. When acid solution was sprayed into the flame, the water content of the interconal gases increased to a maximum of 14%, depending on the rate of spraying, and that of the final products increased to 7·5%. Production of carbon on the burner walls and in the lower cone was again evident and when traces of alkaline impurities were present in the acid solution there was a yellow luminescence, extending well beyond the boundaries of the

FIG. 2. *Variation in $H_2SO_4$ content of combustion products with amount of $H_2SO_4$ or $SO_3$ added to Bunsen-type flame.*




*A*, $H_2SO_4$. *B*, $SO_3$.

FIG. 3. *Equilibrium degree of dissociation of $SO_3$ at various temperatures.*




(uncontaminated) flame. The upper cone remained clear. These phenomena have been described and discussed in detail by Arthur and Littlejohn (*Nature*, 1952, **169**, 288).

The results of the dew-point measurements on the interconal gases and on the final combustion products are shown in Table 2. The figures in column 4 were derived from those in

TABLE 2. *Dew-point and equivalent $H_2SO_4$ concentration of interconal and final products of combustion.*

| $H_2SO_4$ introduced (% by vol.) | Interconal gases | | Upper cone | | |
|---|---|---|---|---|---|
| | Dew-point | $H_2SO_4$, % | $H_2SO_4$, Initial concn., % | Dew-point of products | $H_2SO_4$ in products, % |
| 0·023 | 146° | 0·0043 | 0·0011 | 74° | 0·0008 |
| 0·040 | 164 | 0·0072 | 0·0018 | 108 | 0·0017 |
| 0·070 | 166 | 0·0076 | 0·0019 | 110 | 0·0018 |
| 0·082 | 170 | 0·0081 | 0·0020 | 114 | 0·0020 |
| 0·120 | 177 | 0·0110 | 0·0025 | 116 | 0·0022 |

column 3 and the volumes of the interconal gases and the air entering the upper diffusion flame. The accuracy of dew-point measurements, over the temperature range encountered, is ±2° and the sulphuric acid concentrations in the final column are estimated to be within ±0·0001%.

## DISCUSSION

We consider first the behaviour of sulphuric acid in the Bunsen-type flame. Fig. 2 shows that the amount of sulphuric acid in the combusion products is 10—15% of that added to the flame. Now, the decomposition of sulphuric acid produces sulphur dioxide, and

Dooley and Whittingham (*loc. cit.*) have previously shown that oxidation of the latter to sulphur trioxide occurs in diffusion and pre-mixed flames to an extent which depends on the concentration of dioxide, the fuel : air ratio, and the nature of the fuel burned. To obtain the true degree of decomposition of sulphuric acid it is therefore necessary to subtract from the measured acid content of the gases that amount of acid arising from re-oxidation of sulphur dioxide in and above the outer cone. This can be obtained from the lower curve in Fig. 2, and Table 3 shows how the true degree of decomposition varies with the initial

TABLE 3. *Relation between the degree of decomposition and the initial concentration of acid.*

| | | | | |
|---|---|---|---|---|
| $H_2SO_4$ added, % | 0·0110 | 0·0176 | 0·0322 | 0·0351 |
| Decompn., % | 94·1 | 96·5 | 97·7 | 97·8 |

concentration of acid. It is evident that the decomposition process is almost of first-order with respect to sulphuric acid concentration. The temperature of the flame, when contaminated with acid, was calculated to be 1290°, and from the flame dimensions and the rates of flow of town's gas and air the overall time of passage of a volume element of mixture through the complete flame zone was estimated at $5 \times 10^{-2}$ sec. It is of interest to compare the above values for the degree of decomposition with the equilibrium degree of dissociation of sulphur trioxide according to the system $2SO_3 \rightleftharpoons 2SO_2 + O_2$. Fig. 3, based on the work of Bodenstein and Pohl (*Z. Elektrochem.*, 1905, **11**, 373), shows the amount of trioxide existing under equilibrium conditions over the range 1000—1500°. It is seen that for gases containing 10% of oxygen the degree of dissociation at 1290° is 99·0%.

The results on separated flames (Table 2) show that about 80—90% of the acid introduced is decomposed in the inner cone (calc. flame temperature 1470°), where partial combustion of the hydrocarbons and hydrogen occurs, whereas the further decomposition in the upper, diffusion flame is very much less.

Attention can now be given to the various processes involved when acid spray is injected into the flames. These are : (i) evaporation of acid droplets; (ii) dissociation of acid vapour into sulphur trioxide and water; and (iii) further dissociation of sulphur trioxide into dioxide and oxygen.

Before (ii) and (iii) are considered in detail it is necessary to estimate the time required for the initial process of evaporation. The rate of evaporation of single drops in still and in moving air streams of varying temperature has been studied by several workers (Frössling, *Z. Geophysik*, 1938, **52**, 170; Sjenitzer, Inst. Mech. Engrs. " General Discussion on Heat Transfer," 1951, Part I; E. G. Richardson, *ibid.*), but in the present system the experimental conditions are very dissimilar, as a cloud of drops is heated by radiation from a flame front. Detailed information on the drop-size distribution curve, which is of more significance than the average drop diameter, for the type of spray burner used, has not yet been obtained by us. Initial measurements indicate that the maximum droplet size is of the order of 100 $\mu$ although these large drops are but few in number and only occur with the strongest acid solutions. It is believed that the carbon " streamers " observed when spraying concentrated acid into the flame are due to rapid reactions between the hydrocarbons in the flame and incompletely evaporated drops. Calculations show that for droplets of 1-$\mu$ diameter the time of evaporation is approximately $10^{-3}$ sec. and is less than the time of passage of a volume element of mixture from the burner-mouth to the flame-front of the inner cone.

Bodenstein and Katayama (*Z. Elektrochem.*, 1909, **15**, 354) obtained the following relation for the variation in dissociation of sulphuric acid vapour with temperature :

$$\log K = -5000/T + 0{\cdot}75 \log T - 0{\cdot}00057T + 4{\cdot}086$$

where $K = [SO_3][H_2O]/[H_2SO_4]$

At flame temperatures, the rate of dissociation is extremely fast and it is unlikely that any undissociated sulphuric acid passes through the flame. It is evident from the

results that the further decomposition of sulphur trioxide is also an extremely fast process and there are various possibilities for the reaction mechanism. The bimolecular reactions $2SO_3 \longrightarrow 2SO_2 + O_2$, either in the gas phase or surface-catalysed, can be eliminated because of the high activation energies required ($\lessdot$44 kcal. and $\simeq$25 kcal. respectively) and the absence of surfaces in contact with the flame. Moreover, the experimental results indicate a first-order reaction with respect to sulphur trioxide.

Consideration of the various molecular and atomic species present in the flames provides the following possibilities :

$$SO_3 + M = SO_2 + X \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where M = CO, $H_2$, or $CH_4$

$$SO_3 + H = OH + SO_2 + 18 \text{ kcal.} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Little information exists on the rate of the homogeneous reactions between sulphur trioxide and other molecular species, although it is known that when M = CO, the equilibrium concentration of trioxide according to $CO + SO_3 \rightleftharpoons CO_2 + SO_2$ is extremely small at temperatures exceeding 1000° (Johnstone, Univ. Illinois, Eng. Exp. Stn. Circ. No. 20, Nov., 1929). By analogy with other free-radical reactions, the activation energy of process (2) is likely to be low, of the order of 5—6 kcal., and recent work (Linnett and Pickering, *Trans. Faraday Soc.*, 1951, **47**, 1101) shows that the concentration of hydrogen atoms in hydrocarbon flames may be quite high. Arthur and Littlejohn (*loc. cit.*) recently suggested that the conditions existing in the inner cone of separated flames are particularly conducive to a high concentration of hydrogen atoms.

On the assumption that a partial pressure of hydrogen atoms of 1 mm. exists in the inner cone of pre-mixed town's gas flames—a not unreasonable estimate—calculation shows that 95% of the sulphur trioxide is destroyed in *ca.* $10^{-3}$ sec. The low degree of dissociation of trioxide in the upper cone of the separated flames examined can be mainly attributed to the low temperature of the flame, which contained a large excess of air, and the low concentration of hydrogen atoms likely to occur. It is not possible to state to what extent sulphur dioxide was re-oxidised in this flame, and this matter is under investigation.

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## 635. *Insecticidal Activity and Chemical Constitution. Part V.* Synthesis of Some Di-p-chlorophenylalkanes.*

By E. John Skerrett and D. Woodcock.

A number of di-*p*-chlorophenylalkanes have been prepared in order to examine a suggestion that the toxicity of DDT is in some way associated with the presence of strongly electron-attracting groups in the aliphatic portion of the molecule.

An improved synthesis of 1 : 1-di-*p*-chlorophenylpropan-2-one is described.

Previous investigations of the relation of chemical structure with insecticidal potency in the DDT series have largely been concerned with altering nuclear substituents or varying the nature and number of the halogen atoms in the aliphatic portion of the molecule. In

* Part IV, *J.*, 1952, 2806.

our earlier publications (*J.*, 1950, 2718; 1952, 2804, 2806) analogues (I; R = Cl or Me, R′ = Cl, Me, or OH) which were isosteric with DDT were synthesised but were found not to be contact insecticides against the grain weevil (*Calandra granaria* L.) which readily succumbs to DDT. This led to the suggestion that the high insecticidal activity of DDT might be in some way associated with the presence of strongly electropositive groups in the aliphatic portion of the molecule. Valuable support for this hypothesis lay in the high activity reported by Hass (*Agric. Chemicals*, 1949, **4**, No. 6, p. 67) for the nitro-compounds (II; R = H, R′ = Me, and R = H, R′ = Et) though the DDT analogue (III), containing the highly electron-attracting $CF_3$ group, had been shown by Kirkwood and Dacey (*Canad. J. Res.*, 1946, **24**, *B*, 69) to possess only 1% of the toxicity of DDT. This is probably because of an alteration in lipoid solubility since Kirkwood and Phillips (*J. Pharmacol.*, 1946, **87**, 375) have shown that this compound is not stored in the perirenal fat of rats.

$(p\text{-}C_6H_4R)_2\cdot CMe_2R'$ (I) $\quad (p\text{-}C_6H_4Cl)_2CH\cdot CRR'\cdot NO_2$ (II) $\quad (p\text{-}C_6H_4Cl)_2CH\cdot CF_3$ (III)

$(p\text{-}C_6H_4Cl)_2CH\cdot CHR\cdot NR'_2$ (IV) $\quad (p\text{-}C_6H_4Cl)_2CH\cdot CHR\cdot NR'_3{}^+ X^-$ (V)

The present work was undertaken with a view to examine more fully this electronic interpretation of the high insecticidal potency of DDT.

Whilst the quaternary ammonium cation is highly electron-attracting, quaternary ammonium salts might be expected to be too polar to be readily lipoid-soluble. However, Cowan and Walter (*J. Physiol.*, 1937, **91**, 101) have shown that such an ion can produce repetitive impulses in sciatic-nerve preparations of frogs, in a manner similar to that produced in cray-fish motor neurons by DDT (Welsh and Gordon, *J. Cell. Comp. Physiol.*, 1947, **30**, 147). Moreover, Tattersfield and Gimingham (*Ann. Appl. Biol.*, 1927, **14**, 217) have shown that certain tetramethyl- and tetraethyl-ammonium salts possess some contact activity against *Aphis rumicis* L. The quaternary iodides (V; R = H or Me, R′ = Me, X = I) were therefore prepared, from the corresponding bases (IV; R = H or Me, R′ = H), and the chloride (V; R = R′ = Me, X = Cl) was obtained by the usual method. These salts and the corresponding bases are not contact insecticides against *Calandra granaria*. Attempts were also made to prepare the corresponding triethyl-ammonium iodides (V; R = H or Me, R′ = Et, X = I) in view of the reported superiority of tetraethyl- over tetramethyl-ammonium salts (Cowan and Walter, *loc. cit.*). Though the tertiary amines were isolated, no quaternary salt formation was observed, and an examination of atomic models indicates that such quaternary salts would be seriously sterically hindered.

Whilst the insecticidal potencies of the nitropropane and nitrobutane (II; R = H, R′ = Me and Et respectively) have been found to be 2·18 and 0·78, relative to DDT, the nitroethane (II; R = R′ = H) had no action on *Calandra granaria*. Successive substitution of chlorine atoms, forming the compounds (II; R = H, R′ = Cl, and R = R′ = Cl), also failed to produce insecticidal activity, which is significant in view of the close spatial and electrical similarity between the dichloro-compound and DDT.

To prepare the monochloro-compound (II; R = H, R′ = Cl) it was necessary to add a solution of the sodium salt of the *aci*-form of the nitroethane (II; R = R′ = H) to excess of chlorine in dioxan, since direct chlorination of the *aci*-salt would be expected to give a product contaminated with both the original material and with the dichloro-compound (cf. Meyer and Tscherniak, *Annalen*, 1876, **180**, 112, 123). The latter was readily prepared by chlorination in dioxan–methyl alcohol in the presence of excess of sodium hydroxide.

Chlorination of the nitropropane (II; R = H, R′ = Me) in dioxan in the presence of sodium methoxide formed 1 : 1-di-*p*-chlorophenyl-2-nitro-2-propyl chloride (II; R = Cl, R′ = Me) which possessed insecticidal activity of the same order as the parent compound. Whilst there is a slight increase in the toxicity of (II; R = Cl, R′ = Me)

over that of (II; R = H, R′ = Me), as would be expected if the presence of electrophilic groups increases insecticidal potency, the inactivity of the compounds (II; R = H, R′ = Cl, and R = R′ = Cl) throws some doubt on the validity of this suggestion. It is possible however that there is an optimum electronic density about the second carbon atom in the aliphatic portion of the molecule, above which there is an adverse effect on lipoid solubility and hence on cuticular penetration. The effect of the substitution of the methyl group in the non-toxic nitroethanes (II; R = R′ = H and R = Cl, R′ = H) to form the toxic molecules (II; R = H, R′ = Me, and R = Cl, R′ = Me) respectively may then be explained in terms of higher fat-solubility. The possibility that the methyl group plays some important steric rôle fails to explain the inactivity of the compound (II; R = Cl, R′ = H) which is isosteric with the highly toxic (II; R = Me, R′ = H).

## Experimental

M. p.s are uncorrected. Microanalyses for carbon and hydrogen are by Drs. Weiler and Strauss, Oxford.

2 : 2-*Di*-p-*chlorophenylethylamine*.—A solution of 1 : 1-di-*p*-chlorophenyl-2-nitroethane (3·0 g.; prepared in 58% yield by Müller's method, U.S.P. 2,397,802) in ethyl alcohol (20 ml.) was shaken in hydrogen for 8 hours at room temperature and pressure in the presence of Raney nickel until there was no further uptake. After filtration, the solvent was removed, the residue taken up in ether, and the solution extracted three times with 8N-hydrochloric acid. The combined extracts were basified with 10N-sodium hydroxide, and the *amine* isolated with ether as a colourless viscous liquid (2·1 g., 78%), b. p. 158—162°/1·2 mm. (Found: Cl, 26·0. $C_{14}H_{13}NCl_2$ requires Cl, 26·7%).

The *hydrochloride* was prepared in ether and purified by repeated precipitation from ethyl alcohol by ether as needles, m. p. 227—228° [Found: Cl, 34·7 (total), 11·7 (ionisable). $C_{14}H_{14}NCl_3$ requires Cl, 35·2 (total), 11·7% (ionisable)]. The acetate was a viscous oil.

N-(2 : 2-*Di*-p-*chlorophenylethyl*)*trimethylammonium Iodide*.—The above amine (1 g.), methyl alcohol (5 ml.), methyl iodide (5 ml.) and excess of anhydrous potassium carbonate were refluxed for 16 hours. The hot solution was filtered and the quaternary *salt* purified by repeated precipitation with dry ether from ethyl alcohol. It formed prismatic plates, m. p. 218—219° (Found: I, 28·8. $C_{17}H_{20}NCl_2I$ requires I, 29·1%).

2-*Chloro*-1 : 1-*di*-p-*chlorophenyl*-2-*nitroethane*.—Sodium hydroxide (0·14 g.) in water (5 ml.) was added to a solution of 1 : 1-di-*p*-chlorophenyl-2-nitroethane (1 g.) in a mixture of dioxan (5 ml.) and methyl alcohol (5 ml.) at 0°, and the whole then added dropwise to dioxan (15 ml.) stirred at 0° during the passage of chlorine (cf. Meyer and Tscherniak, *Annalen*, 1876, **180**, 112, 126). Stirring and chlorination were continued for 5 minutes after the final addition and then the solution was evaporated *in vacuo*. The residue was extracted with ether, the extract washed with water and sodium hydrogen carbonate solution and dried, and the solvent removed. The residual *compound* crystallised from aqueous methyl alcohol in rhombic prisms (0·75 g., 67%), m. p. 86—87° (Found: C, 51·4; H, 3·1; Cl, 32·9. $C_{14}H_{10}O_2NCl_3$ requires C, 50·8; H, 3·1; Cl, 32·2%).

1 : 1-*Dichloro*-2 : 2-*di*-p-*chlorophenyl*-1-*nitroethane*.—A solution of the nitro-compound (II; R = R′ = H) (1 g.) in a mixture of dioxan (10 ml.) and methyl alcohol (7 ml.) was cooled to 0° and stirred during the addition of sodium hydroxide (0·4 g.) in water (3 ml.). A rapid stream of chlorine was passed through the solution for 0·5 hour and then the solvents were removed by distillation under reduced pressure. The *product*, isolated with ether, crystallised from methyl alcohol in well-formed octahedra (0·7 g., 57%), m. p. 76—78° (Found: C, 46·0; H, 2·5; Cl, 38·7. $C_{14}H_9O_2NCl_4$ requires C, 46·0; H, 2·5; Cl, 38·9%).

2-*Chloro*-1 : 1-*di*-p-*chlorophenyl*-2-*nitropropane*.—1 : 1-Di-*p*-chlorophenyl-2-nitropropane (12·4 g.) dissolved in anhydrous dioxan (120 ml.) was cooled to 0° and stirred for 1 hour in the presence of sodium methoxide (from 1·6 g. of sodium), during the passage of chlorine. The solvent was removed under reduced pressure, the residue extracted with ether, and the extract washed twice with water and saturated sodium hydrogen carbonate solution and dried ($Na_2SO_4$). Removal of the ether gave a *product* which crystallised from methyl alcohol in monoclinic prisms, m. p. 96—97° (Found: C, 52·4; H, 3·6; Cl, 30·9. $C_{15}H_{11}O_2NCl_3$ requires C, 52·3; H, 3·5; Cl, 30·9%). Attempts to prepare this product by direct chlorination in carbon tetrachloride solution at 0° resulted in recovery of the original nitro-compound.

1 : 1-*Di*-p-*chlorophenyl*-2-*propylamine*.—(*a*) A solution of 1 : 1-di-*p*-chlorophenyl-2-oximino-

propane (3·5 g.; see below) in glacial acetic acid (130 ml.) was stirred for 12 hours and 3% sodium amalgam (350 g.) added in small portions (cf. Goldschmidt, *Ber.*, 1886, **19**, 3232; 1887, **20**, 728). Crushed ice (1 kg.) was added, the mixture made alkaline with 10N-sodium hydroxide, and the product extracted with ether. Removal of the solvent and distillation of the residue gave the *amine* as a viscous liquid (3·0 g., 90%), b. p. 180—182°/0·8 mm., which slowly solidified. Crystallisation from light petroleum (b. p. 40—60°) gave rectangular prisms, m. p. 90—91° (Found: C, 64·5; H, 5·4; N, 5·0; Cl, 25·1. $C_{15}H_{13}NCl_2$ requires C, 64·3; H, 5·4; N, 5·0; Cl, 25·3%).

(*b*) An ethyl-alcoholic solution (35 ml.) of the nitropropane (II; R = H, R′ = Me) (5·0 g.) was shaken for 12 hours in hydrogen at room temperature and 6 atmospheres in the presence of Raney nickel. The product, isolated as described in (*a*) above, was distilled (b. p. 175—180°/0·8 mm.) and crystallised from light petroleum (b. p. 40—60°) in rectangular prisms (3·6 g., 80%), m. p. 90—91°, undepressed by admixture with the product from (*a*). Hydrogenation at atmospheric pressure lowered the yield to 50%. The *acetate* crystallised from ethyl alcohol in minute prisms, m. p. 175—176° (Found: C, 63·5; H, 5·3. $C_{17}H_{17}ONCl_2$ requires C, 63·3; H, 5·3%). The *hydrochloride*, prepared in ether and purified as described for the hydrochloride of the ethylamine, formed needles, m. p. 261—262° [Found: Cl, 10·8. $C_{15}H_{14}NCl_3$ requires Cl (ionisable), 11·2%].

N-(1 : 1-*Di*-p-*chlorophenyl*-2-*propyl*)*trimethylammonium Iodide*.—The foregoing amine (1·0 g.), methyl iodide (5 ml.), methyl alcohol (5 ml.), and excess of anhydrous potassium carbonate were heated under reflux for 16 hours. The *iodide*, isolated as previously described, formed stout rectangular prisms which decomposed at 180—200° (Found: C, 49·7; H, 5·2; Cl, 15·2. $C_{18}H_{22}NCl_2I$ requires C, 48·0; H, 4·9; Cl, 15·8%). Attempts to prepare the corresponding triethylammonium iodide resulted in the formation of N-(1 : 1-*di*-p-*chlorophenyl*-2-*propyl*)*diethylamine*, b. p. 180—190°/0·5 mm. (Found: Cl, 21·0. $C_{19}H_{23}NCl_2$ requires Cl, 21·1%), which did not react further with ethyl iodide in ether.

1 : 1-*Di*-p-*chlorophenylpropane*-1 : 2-*diol*.—Ethyl lactate (11·8 g.) in anhydrous ether (35 ml.) was added dropwise with stirring to an ice-cooled solution of *p*-chlorophenylmagnesium bromide, prepared from magnesium turnings (7·3 g.) and *p*-bromochlorobenzene (57·5 g.) in anhydrous ether (150 ml.). The mixture was kept at 0° for 66 hours and then cautiously decomposed at this temperature with 2N-sulphuric acid. After being washed with 1% sodium hydrogen sulphite solution, water, and saturated sodium hydrogen carbonate solution, the ethereal layer was dried ($Na_2SO_4$). Removal of the solvent and distillation of the residue gave a fraction (19·2 g., 65%), b. p. 190—200°/1·0 mm., which solidified and crystallised from light petroleum (b. p. 60—80°) in rhombs, m. p. 96—97° (Found: C, 60·5; H, 4·7; Cl, 24·1. $C_{15}H_{14}O_2Cl_2$ requires C, 60·6; H, 4·7; Cl, 23·9%). This *diol* gave a *diacetate*, large rhombic prisms (from methyl alcohol), m. p. 131—132° (Found: C, 60·0; H, 4·6. $C_{19}H_{18}O_4Cl_2$ requires C, 59·8; H, 4·7%).

1 : 1-*Di*-p-*chlorophenylpropan*-2-*one*.—(*a*) This ketone, earlier reported as a crystalline solid, m. p. 52—53° (Skerrett and Woodcock, *loc. cit.*; cf. Erlenmeyer, Bitterli, and Sorkin, *Helv. Chim. Acta*, 1948, **31**, 466) has now been prepared by a much improved procedure based on Bowman's method (*J.*, 1950, 322). A mixture of ethyl malonate (48 g.), ethyl alcohol (14 ml.), and benzene (60 ml.) was slowly added to a vigorously stirred hot mixture of magnesium turnings (7·2 g.), benzene (22 ml.), ethyl alcohol (2 ml.), and carbon tetrachloride (0·1 ml.). When solution of the magnesium was complete the benzene–ethyl alcohol azeotrope was removed. Di-*p*-chlorophenylacetyl chloride (44·2 g.) was then added and the mixture heated under reflux for 3 hours and poured into water. The benzene layer was separated, washed with 2N-sulphuric acid and water, and evaporated to dryness on a water-bath under reduced pressure. The residual oil was heated under reflux for 6 hours with propionic acid (120 ml.) and concentrated sulphuric acid (1·2 ml.) and then for 1—2 days after the addition of 4N-sulphuric acid (12 ml.). Crystalline sodium acetate (22 g.) was then added and volatile acids were removed by distillation under reduced pressure. After the addition of ice to the residue, organic matter was extracted with ether, and the ethereal solution washed with 1% sodium hydroxide solution and water and dried. The solvent was removed and the residue distilled as a viscous liquid (35·5 g., 86%), b. p. 182—184°/1 mm., which slowly solidified. Crystallisation from methyl alcohol yielded monoclinic prisms, m. p. 52—53° (Found: C, 64·6; H, 4·3. Calc. for $C_{15}H_{12}OCl_2$: C, 64·5; H, 4·3%). When the method of Erlenmeyer *et al.* (*loc. cit.*) was used the distilled product solidified but was almost invariably contaminated with di-*p*-chlorophenylpropan-2-one, m. p. 146—147°, and ethyl di-*p*-chlorophenylacetate, m. p. 88—89°, both m. p.s being undepressed by admixture with the appropriate authentic specimens.

(*b*) 1 : 1-Di-*p*-chlorophenylpropane-1 : 2-diol (1·0 g.) was heated with potassium hydrogen sulphate (2·0 g.) for 70 minutes at 140°. The mixture was cooled and extracted with ether, the ethereal layer being washed with water and then saturated sodium hydrogen carbonate solution, and dried ($Na_2SO_4$). Removal of the solvent and distillation of the residue gave a viscous liquid (0·9 g., 99%), b. p. 180—190°/2 mm., which slowly solidified. It crystallised from methyl alcohol in prisms, m. p. 50—51° undepressed by admixture with the product obtained as in (*a*). An attempt to prepare this ketone by an adaptation of Stoermer's method (*Ber.*, 1906, **39**, 2288), *viz.*, by heating 1 : 1-di-*p*-chlorophenylpropane-1 : 2-diol with acetic anhydride under reflux for 3 hours, gave only the diacetyl derivative of the diol.

The oxime prepared in the usual way crystallised from ethyl alcohol in slender rectangular prisms, m. p. 173—174°. Erlenmeyer *et al.* (*loc. cit.*) reported m. p. 170—171°. The 2 : 4-*dinitrophenylhydrazone* crystallised from ethyl alcohol–ethyl acetate in rhombs, m. p. 168—169° (Found : Cl, 15·8. $C_{21}H_{16}O_4N_4Cl_2$ requires Cl, 15·5%).

1 : 1-*Di*-p-*chlorophenylpropan*-2-*ol*.—The above ketone (8·2 g.) in anhydrous ether (25 ml.) was added to a solution of lithium aluminium hydride (0·8 g.) in anhydrous ether (50 ml.) at such a rate as to produce gentle ebullition. The mixture was set aside for 17 hours, cooled in ice-water, and cautiously decomposed by the addition of water (2 ml.) and then concentrated hydrochloric acid. The ethereal layer was separated, washed with 2N-hydrochloric acid and water and dried ($Na_2SO_4$). The solvent was removed and the residue on distillation yielded a viscous liquid (7·9 g., 95%), b. p. 190—195°/1·7 mm., which slowly solidified. The *alcohol* crystallised from light petroleum (b. p. 40—60°) in monoclinic prisms, m. p. 92—93° (Found : C, 64·1; H, 4·9; Cl, 25·0. $C_{15}H_{14}OCl_2$ requires C, 64·1; H, 5·0; Cl, 25·2%). The *acetate*, prepared in the usual manner, crystallised from methyl alcohol in rhombic prismatic plates, m. p. 94—95° (Found : C, 63·1; H, 5·0. $C_{17}H_{16}O_2Cl_2$ requires C, 63·1; H, 5·0%).

Attempts to prepare 1 : 1-di-*p*-chlorophenyl-2-propyl chloride by reaction of the above alcohol with thionyl chloride in pyridine gave only unchanged starting material.

2 : 2-*Di*-p-*chlorophenylethan*-1-*ol*.—A solution of di-*p*-chlorophenylacetic acid (5 g.) in anhydrous tetrahydrofuran (30 ml.) was added to a solution of lithium aluminium hydride (2 g.) in anhydrous ether (100 ml.), and the mixture refluxed for 7 hours (cf. Nystrom and Brown, *J. Amer. Chem. Soc.*, 1947, **69**, 2548). The reaction mixture was cooled in ice-water and decomposed by the addition of methyl alcohol (2 ml.) and concentrated hydrochloric acid, and the product isolated as for the preceding alcohol. It was a viscous liquid (4·3 g., 91%), b. p. 170—180°/2 mm., which solidified and crystallised from light petroleum (b. p. 60—80°) in massive rhombs, m. p. 97·5—98° (Found : C, 63·0; H, 4·5. Calc. for $C_{14}H_{12}OCl_2$ : C, 62·9; H, 4·5%). Some 12 months after this compound had been prepared, Grummitt, Arters, and Stearns (*ibid.*, 1951, **73**, 2548) reported a 39% yield and m. p. 98·5—99·5°. The *acetate* crystallised from methyl alcohol in aggregates of prismatic plates, m. p. 67—68° (Found : C, 62·2; H, 4·4; Cl, 22·8. $C_{16}H_{14}O_2Cl_2$ requires C, 62·2; H, 4·5; Cl, 22·9%). Attempts to prepare a phenylurethane failed.

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## 636. *Humulinone: its Alleged Occurrence in Hops.*

By M. Verzele and F. Govaert.

Recently Cook and Harris (*J.*, 1950, 1873) obtained a strongly acidic compound (p$K$ 2·7), which they called "humulinone," by treatment of the light-petroleum soluble hop resins with either saturated aqueous sodium hydrogen carbonate (giving the sodium salt) or 90% methanolic lead acetate (a mixture of the lead salts of humulone and humulinone then being obtained). These authors claimed that humulinone was present as a constituent of the hop resins, but its existence in hops, in the quantities reported, is incompatible with the results of hop analyses previously reported by us (*Congr. Intern. Inds. Fermentation*, 1947, 279; *Fermentatio*, 1948, **1**, 1).

In the first of our methods the chromatographically purified extract is treated with lead acetate, thus precipitating the α-acid, and the β-acid content is determined by potentiometric titration of the mother-liquor. In the second method the α-acid content is determined polarimetrically and the β-acid content from the total acidity, by difference. The results for α-acid content, obtained by use of the two methods, have always agreed, but this could not be so if humulinone were present as a constituent, for in the first method it would be precipitated with humulone and therefore not measured in the lupulone fraction, whereas in the second method the lupulone content would be augmented by that of humulinone.

If humulinone occurs naturally as a constituent of the hop resins it should be obtained from a methanol–water (1 : 1) solution of hop extract by extraction at pH 3·5, to remove neutral products, humulone (p$K$ 5·4), and lupulone (p$K$ 6·0), followed by adjustment of the pH to <2·0, and re-extraction. No humulinone could, however, be obtained by this method, although our material gave humulinone readily when Cook and Harris's method was used.

Humulinone is strongly retained on silica gel (15 g. of gel retain about 700 mg. of the compound), from which it cannot be eluted completely with benzene but should be removed by ether. If present in hops, humulinone would therefore be retained on the column when chromatographic purification was used. Elution in this way and treatment of the eluate with ether and aqueous sodium hydrogen carbonate gave no humulinone, provided that all humulone had been eluted previously (lead acetate test).

The only remaining possibility is that humulinone is an artefact, produced by oxidation of humulone after extraction from the hops. This is proved to be the case. Pure humulone remains unchanged in pure ether and sodium hydrogen carbonate solution in the absence of oxygen, but if the ether contains peroxides humulinone is obtained in yields of up to 50%. The formation of humulinone can be brought about in pure ether by small additions of perhydrol. The oxidation is catalysed by light petroleum [this catalytic influence of light petroleum on the oxidation of bitter acids is well known (cf. Lundin, *Wallerstein Lab. Comm.*, 1947, **10**, 231)], and traces of this were probably still present in the oils (obtained by evaporation of light petroleum extracts of the hop resins) from which Cook and Harris obtained humulinone.

Examination of several old hop samples indicated that humulinone was not formed during storage; it could not be isolated provided that all oxidising agents were carefully avoided during the treatment of the resin extracts with ether and sodium hydrogen carbonate solution.

Humulinone is not precipitated from methanol by lead acetate (cf. Cook and Harris, *loc. cit.*); indeed normal precipitation of humulone is hampered by the presence of humulinone.

We find that humulinone has no optical activity and there is evidence that it may be a mixture of three isomers.

*Experimental.—Materials.* Pure humulone was obtained by chromatographic purification of hop extract, as described in previous papers from this laboratory; it had m. p. 65—66° (phenylenediamine complex, m. p. 115—117°). The ether used was purified by washing, drying,

and fractionating the commercial material. "Enriched" ether was obtained by four-fold concentration of commercial ether which had been stored for long periods in clear-glass bottles. Sodium hydrogen carbonate solutions were prepared with water which had been boiled.

*Experiments with humulone.* (*a*) Pure humulone (1 g.), pure ether (25 c.c.), and sufficient sodium hydrogen carbonate solution (about 30 c.c.) to fill the flask were kept for 7 days out of contact with air. No apparent change occurred and the optical rotation of the product represented 975 mg. of humulone (humulinone having no optical activity).

(*b*) When the pure ether was replaced by "enriched" ether, carbon dioxide was evolved, and the sodium salt (384 mg.) of humulinone was obtained; this gave humulinone (327 mg.), m. p. 70—74°. No humulone was detected.

(*c*) The use of "enriched" ether and perhydrol (2·5 c.c.) led to only a small yield of humulinone (26 mg.); no humulone could be detected. A smell of fatty acids suggested that the oxidation had proceeded too far.

*Experiments with hop extracts.* The optical rotation of the hop extract indicated 37·2 g./l. of α-acid.

(*a*) When oxygen was rigorously excluded from a mixture of the extract, pure ether, and sodium hydrogen carbonate solution, no humulinone was obtained and the optical rotation showed no appreciable loss of humulone during 7 days.

(*b*) Treatment of the hop extract (25 ml.) with "enriched" ether and sodium hydrogen carbonate solution gave the sodium salt (464 mg., 45%) of humulinone. The residue had no optical activity.

(*c*) Addition of perhydrol (2 c.c.) to the solution as used in (*b*) resulted in a smaller yield of the salt (325 mg.). The residue had a small optical rotation.

(*d*) The yield of humulinone was very variable, the reaction easily proceeding too far. The highest yield was obtained by the addition of a few drops of perhydrol each day to the mixture until humulinone ceased to separate. In this way 2·38 g. of α-acid with 50 c.c. of ether and 50 c.c. of saturated sodium hydrogen carbonate solution gave 1·206 g. (49%) of the sodium salt of humulinone.

(*e*) An extract of hops (15 g.), from which α-acid had been completely removed by lead acetate treatment, did not give any humulinone on treatment with ether and sodium hydrogen carbonate solution.

*Precipitations with lead acetate.* (*a*) Treatment of a solution of α-acid (175 mg.) in methanol (10 c.c.) and water (1·75 c.c.) with 4%-methanolic lead acetate (4·60 c.c.) gave the lead salt (270 mg.) of humulone.

(*b*) When humulinone (100 mg.) was added to α-acid the lead salt (271 mg.) of humulone was again obtained, but (*c*) when the quantity of humulinone was doubled the amount of lead salt isolated fell (to 230 mg.).

(*d*) If the water was replaced by the same quantity of 0·3N-sodium hydroxide (enough to neutralise the humulinone) the yield of lead salt obtained in (*c*) rose to the normal value (268 mg.).

(*e*) Humulinone was not precipitated by lead acetate from methanolic solution.

*Humulinone.* Crude humulinone was adsorbed on a small column of silica gel and the chromatogram was well washed with benzene. The filtrate was concentrated and the residue crystallised about ten times from light petroleum and then from dilute methanol. The product had m. p. 104—105°, p*K* 2·6 (in 50% methanol) (Found: *M*, 378. Calc. for $C_{21}H_{30}O_6$: *M*, 378). The light petroleum mother-liquor yielded a second isomer, m. p. 70—71°, on evaporation. This had p*K* 2·5 (in 50% methanol) (Found: *M*, 381). Elution of the column with ether, removal of the solvent, and crystallisation of the residue from light petroleum–benzene and from dilute methanol gave a third compound, m. p. 182°, p*K* 2·8 (in 50% methanol) (Found: *M*, 380).

UNIVERSITY OF GHENT, BELGIUM. [*Received, March 10th*, 1952.]

## 637. *The Preparation and Stability of Hydroxymaleic Acid (Oxaloacetic Acid).*

By John C. Roberts.

The importance of oxaloacetic acid as an intermediate compound in animal and plant metabolism and its frequent use in biochemical investigations has necessitated a re-examination of the laboratory methods available for its preparation and its stability in the solid state. (The stability of the acid in aqueous solution has been investigated by Krebs, *Biochem. J.*, 1942, **36**, 303.)

Oxaloacetic acid is generally prepared by one of the following methods: (i) Malic acid is oxidised with hydrogen peroxide (Fenton and Jones, *J.*, 1900, **77**, 77). Reproducible results are difficult to obtain, the technique is laborious (necessitating a very large number of ether-extractions), and the yields are not good (Fenton and Jones claim a yield of "about 22%" of the presumably unrecrystallised acid). (ii) Ethyl oxaloacetate is hydrolysed with concentrated hydrochloric acid (Krampitz and Werkmann, *Biochem. J.*, 1941, **35**, 596). These authors do not state the yield. The present author, using a temperature of −20° for cooling the acid hydrolysis liquid, has obtained very poor yields (*ca.* 6% of unrecrystallised acid). It is possible that results are very greatly dependent on the purity of the starting materials (cf. Simon, *Compt. rend.*, 1903, **137**, 856). (iii) The pyridine salt of hydroxymaleic anhydride is decomposed with sulphuric acid (Wohl and Oesterlin, *Ber.*, 1901, **34**, 1144). Use of 12% sulphuric acid yields hydroxymaleic acid; 30% sulphuric acid gives hydroxyfumaric acid (Wohl and Lips, *Ber.*, 1907, **40**, 2294). These observations led Wohl and Claussner (*ibid.*, p. 2308) to the development of a method for preparing the two isomeric acids in a state of high purity but yields are poor. No precise yields are stated by Wohl and Claussner. The present author has obtained 27% of recrystallised hydroxyfumaric acid and 8% of recrystallised hydroxymaleic acid—yields calculated on the pyridine salt.

The following modification of the method of Wohl *et al.* yields very pure hydroxymaleic acid in much improved yield more reliably and less tediously.

*Experimental.—Hydroxymaleic acid.* Powdered tartaric acid (20 g.) is dissolved, at room temperature, in acetic anhydride (44 c.c.) containing concentrated sulphuric acid (0·6 c.c.) in a 100-c.c. flask fitted to a reflux condenser by a ground-glass joint. The solution is refluxed gently for 10 minutes, then cooled to room temperature, and crystallisation of the diacetyltartaric anhydride is induced by "scratching." After 1 hour at room temperature the crystals are filtered off (Whatman, No. 54 paper), pressed, washed with benzene (3 × 10 c.c.), pressed and drained thoroughly and kept in a vacuum over paraffin wax for 1½ hours. The yield of material of m. p. 129° is 23·0—24·2 g. (80—84%).

Diacetyltartaric anhydride (20 g.) is added, at room temperature, to *pure dry* pyridine (40 c.c.), in a thin-walled flask, and the mixture shaken vigorously. A pale green colour quickly develops whereupon glacial acetic acid (12 c.c.) is added, the whole warmed, with continous shaking, in warm (40°) water for 20 seconds, and the flask then immersed in crushed ice. Ice-cold *dry* ether (45 c.c.) is added, and the whole quickly mixed and immediately filtered. The pyridine salt of hydroxymaleic anhydride is pressed thoroughly, washed with absolute alcohol (3 × 8 c.c.), and then with *dry* ether (2 × 8 c.c.). The salt, m. p. 108—109° (decomp.), is very light greenish-brown and is preferably used without delay in the ensuing operation. The yield is somewhat variable (10·4—13·3 g.; average 12·0 g., 67%). (All the operations described in this paragraph must be performed without any delays.)

The pyridine salt of hydroxymaleic anhydride (12 g.) is added to ice-cold concentrated hydrochloric acid (18 c.c.). A dark green solution is produced which, after 15 minutes, begins to deposit dirty-white crystals. The mixture is left at −2° overnight. The crude oxaloacetic acid is filtered off (Whatman, No. 54 filter paper), pressed, washed with ice-cold 7N-hydrochloric acid (3 × 6 c.c.), well pressed, and drained. Finally, it is washed with ice-cold chloroform (3 × 5 c.c.) and again well pressed and drained. The crystals are kept in a vacuum-desiccator over phosphoric oxide and potassium hydroxide pellets for 1½ hours. The yield of crude oxaloacetic acid is 6·55 g. (80%).

The crude acid (6·5 g.) is dissolved in warm (50°) acetone (65 c.c.) and filtered through a plug of acetone-washed cotton-wool in a pre-warmed funnel. To the acetone solution is added, with stirring, warm (50°) chloroform (250 c.c.) and the mixture set aside until crystallisation is complete (about 1 hour). The crystals are filtered off, washed with a mixture (2 × 8 c.c.) of acetone (1 vol.) and chloroform (4 vols.) and dried in a vacuum-desiccator over phosphoric oxide for 1 hour. The product (4·4 g., 67%), m. p. 155°, is free from chlorides and, after having been dried for 6 hours, shows an equivalent of 66·1 (Calc. for $C_4H_4O_5$, as a dibasic acid: equiv., 66·04); the m. p. gradually falls and the equivalent increases—see below.

The overall yield of recrystallised hydroxymaleic acid, calculated on the tartaric acid used, is about 30%.

*Stability.* A sample of hydroxymaleic acid was prepared by the method of Wohl and Claussner (*loc. cit.*), recrystallised from acetone–benzene and stored in a vacuum-desiccator over phosphoric oxide for the first day, and, thereafterwards, in an ordinary desiccator over anhydrous calcium chloride. Alterations in m. p. and equivalent, occurring on storage, are recorded in the Table.

| "Age" (hours) ..... | 0 | 0·5 | 1·75 | 3·5 | 23 | 56 | 80 | 120 |
|---|---|---|---|---|---|---|---|---|
| M. p.[a] ..... | 157° | 157° | 153° | 153° | 152·5° | 146·5° | 146·5° | 144° |
| Equiv.[b] ..... | — | — | — | 66·1 | — | — | — | 66·3 |

[a] M. p.s were determined with the bath heated at about 12°/minute. [b] Equivs. were determined by dissolution in ice-cold water and titration, immediately and rapidly, with 0·1N-sodium hydroxide (phenolphthalein). The first pink colour which persisted for 15 seconds was taken as the end-point.

If the fall of the m. p. and the increase of the equivalent weight are due to decarboxylation, the sample which had been stored for 5 days (equiv. 66·3) contained about 98·8% of hydroxymaleic acid (equiv., 66·04) and 1·2% of pyruvic acid (equiv., 88·06).

A sample of hydroxymaleic acid, m. p. 152—153°, "age" 3 hours, was decarboxylated with "aniline citrate" reagent (Edson, *Biochem. J.*, 1935, **29**, 2083), and the carbon dioxide produced (during 1 hour) was removed in a brisk current of carbon dioxide-free air, absorbed in "Sofnolite," and weighed. A purity of 98·5% was indicated. It appears that values obtained by the decarboxylation method are slightly low.

THE UNIVERSITY, NOTTINGHAM. [*Received, May 5th*, 1952.]

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## 638. *A Synthesis of* (+)-β-*Aminobutyric Acid from* L-*Alanine.*

By K. BALENOVIĆ, D. CERAR, and Z. FUKS.

β-AMINOBUTYRIC ACID was first resolved into its optical antipodes by Fischer and Scheibler (*Annalen*, 1911, **383**, 337) by using a very tedious procedure; the (+)- and the (−)-form showed $[\alpha]_D$ +35·3° and $[\alpha]_D$ −35·2°, respectively.

By application of the Arndt–Eistert reaction to (−)-1-diazo-3-phthalimidobutan-2-one, prepared from L-alanine (cf. *Experientia*, 1947, **3**, 369), (+)-β-aminobutyric acid, $[\alpha]_D$ +38·8°, has been obtained in five stages, in an overall yield of 38% (based on L-alanine).

*Experimental.*—*N-Phthaloyl-L-alanyl chloride.* *N*-Phthaloyl-L-alanine (3 g.; $[\alpha]_D^{18}$ −17·5°) (*Ber.*, 1907, **40**, 489) and thionyl chloride (6 c.c.) were heated for 1 hour at 60°; the excess of thionyl chloride was then removed under reduced pressure, the residue distilled, and the distillate (2·94 g., 92%), b. p. 186—192°/15 mm., 135°/1 mm., crystallised from light petroleum (b. p. 30—50°). The chloride had m. p. 38°, $[\alpha]_D^{17}$ −36·4° ±0·5° (*c*, 0·20 in benzene) (Found: C, 55·9; H, 3·3. Calc. for $C_{11}H_8O_3NCl$: C, 55·6; H, 3·4%). Gabriel (*Ber.*, 1908, **41**, 247) reported m. p. 73° for the inactive form.

(−)-1-*Diazo-3-phthalimidobutan-2-one.* The chloride (6 g.) was dissolved in benzene (25 c.c.) and added to an ethereal solution of diazomethane (500 c.c.; obtained from 35 g. of nitrosomethylurea). After 24 hours the mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in the minimum quantity of dry ethyl acetate and precipitated with light petroleum. The (−)-1-*diazo-3-phthalimidobutan-2-one* (5·2 g., 85·2%), m. p. 88°, obtained was recrystallised from ethyl acetate–light petroleum; the m. p. remained unchanged, $[\alpha]_D^{18}$ −69·3° ±1° (*c*, 0·48 in ethyl acetate) (Found: C, 59·5; H, 3·7. $C_{12}H_9O_3N_3$ requires C, 59·3; H, 3·7%).

*Methyl* (+)-β-*phthalimidobutyrate*. A freshly prepared alkali-free suspension of silver oxide (obtained from 0·5 g. of silver nitrate) was gradually added in four portions during 3 hours to a refluxing solution of the diazo-ketone (2·7 g.) in methanol (27 c.c.). The hot solution was treated with charcoal, filtered, and evaporated to dryness. The residue was extracted several times with light petroleum, the solvent removed from the combined extracts, and the product (1·8 g., 65%) repeatedly crystallised from light petroleum and finally sublimed at 110°/0·01 mm. *Methyl* (+)-β-*phthalimidobutyrate* had m. p. 38°, $[\alpha]_D^{16}$ +26·3°±1° (*c*, 0·26 in benzene) (Found: C, 62·9; H, 5·4. $C_{13}H_{13}O_4N$ requires C, 63·2; H, 5·3%).

(+)-β-*Aminobutyric acid*. A solution of the methyl ester (2 g.) in glacial acetic acid (5 c.c.) and 50% hydriodic acid (6 c.c.) was heated under reflux for 7 hours. After cooling, the phthalic acid was filtered off and the filtrate evaporated to dryness under reduced pressure. The residue was dissolved in water, the solution extracted with ether (3 × 50 c.c.), and the water layer again evaporated to dryness. The crystalline hydriodide of (+)-β-aminobutyric acid was dissolved in water (400 c.c.) and passed through a column of Amberlite IR-4B (20—50 mesh; 15 g.) at a flow rate of 200 c.c./hour. The column was washed with distilled water (200 c.c.), and the washings were evaporated *in vacuo*. (+)-β-Aminobutyric acid (750 mg., 89%), m. p. 212°, was twice recrystallised from absolute methanol (the m. p. remained unchanged); it then had $[\alpha]_D^{18}$ +38·8°±1° (*c*, 0·48 in water), $[\alpha]_D^{19}$ +37·07°±1° (*c*, 6·0 in water) (Found: C, 46·3; H, 8·9. Calc. for $C_4H_9O_2N$: C, 46·6; H, 8·80%). Fischer and Scheibler (*loc. cit.*) reported $[\alpha]_D^{20}$ +35·3°±0·2° (*c*, 11·41 in water).

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ZAGREB, YUGOSLAVIA.

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## 639. *Mannich Bases of Acylaminomalonates, and a Synthesis of* DL-*Aspartic Acid.*

By R. O. ATKINSON.

IN connection with an investigation into possible methods of preparing certain β-substituted alanines, Mannich bases of ethyl acetamidomalonate and ethyl formamidomalonate were prepared. Butenandt and Hellmann (*Z. physiol. Chem.*, 1949, **284**, 168) prepared several of these compounds, using secondary amines of higher molecular weight [piperidine, morpholine, etc.], but they were unable to prepare the dimethylamino-compounds in crystalline form.

A procedure is described for preparing the dimethylaminomethyl compounds. The bases slowly decomposed when kept, but the readily prepared methiodides were quite stable.

Unlike Butenandt's (*loc. cit.*) piperidino-compound which, on acid hydrolysis, gave β-piperidylalanine, the dimethylamino-compounds gave glycine under the same conditions.

As expected, the esters would not react with sodium cyanide, the formation of an ethylenic intermediate being impossible. Snyder and Eliel (*J. Amer. Chem. Soc.*, 1948, **70**, 1073) and Snyder and Brewster (*ibid.*, 1949, **71**, 1058) have shown that in similar cases the necessary activation may be brought about by quaternisation.

Condensation of the methiodide with sodium cyanide proceeded vigorously in aqueous solution, and acid hydrolysis of the product gave aspartic acid in 71% yield.

*Experimental.—Diethyl* α-*acetamido*-α-*dimethylaminomethylmalonate*. 33% Dimethylamine solution [13·5 c.c., 0·1 mole] was treated at 0° with glacial acetic acid (15 c.c.). Diethylacetamidomalonate [21·7 g., 0·1 mole] and 40% formaldehyde solution [8·2 c.c.] were added and, after 30 minutes at room temperature, the mixture was cooled to −10° and made alkaline by slow addition of 20% sodium hydroxide solution. The Mannich *base* separated as white crystals (20 g., 73%), m. p. 50—51° (uncorr.) (Found: N, 10·1. $C_{12}H_{22}O_5N_2$ requires N, 10·2%).

The Mannich base (27·4 g., 0·1 mole) was dissolved in ethyl alcohol (60 c.c.), and methyl iodide (16 g., 0·11 mole) added; the *methiodide*, which crystallised spontaneously, was filtered

off after 30 minutes, washed with ether, and dried in air at 80° (19·6 g., 47·1%). It had m. p. 384° (uncorr.) (Found : N, 6·75; $I^-$, 31·1. $C_{12}H_{25}O_5N_2I$ requires N, 6·95; $I^-$, 30·5%).

*Diethyl α-dimethylaminomethyl-α-formamidomalonate.* Diethyl formamidomalonate (20·3 g., 0·1 mole) treated as described above gave the Mannich *base* as broken white plates (26 g., 100%), m. p. 77—78° (uncorr.) (Found : N, 10·6. $C_{11}H_{20}O_5N_2$ requires N, 10·7%). The base (26 g., 0·1 mole) with methyl iodide (16 g., 0·011 mole) gave the *methiodide* (19 g., 47·5%), m. p. 318—319° (uncorr.) (Found : N, 6·6; $I^-$, 31·2. $C_{12}H_{23}O_5N_2I$ requires N, 6·6; $I^-$, 31·7%).

DL-*Aspartic acid.* Diethyl α-dimethylaminomethyl-α-formamidomalonate methiodide (20·1 g., 0·05 mole) was added to a solution of sodium cyanide (4·9 g., 0·1 mole) in water (25 c.c.), and the mixture was warmed on a steam-bath ; after a few seconds a vigorous reaction started and the solution became dark red. It was cooled for 5 minutes (the reaction becoming less vigorous), heated on a steam-bath for 3 hours, and then evaporated to dryness *in vacuo*. The residue was dissolved in hydrochloric acid (120 c.c.) and heated on a steam-bath for 6 hours. Evaporation to dryness *in vacuo*, and treatment of the alcoholic extract with pyridine gave the crude amino-acid, which was recrystallised from hot water. The yield was 4·7 g. (71%) (Found : C, 36·0; H, 5·2; N, 10·6. Calc. for $C_4H_7O_4N$ : C, 36·1; H, 5·3; N, 10·5%). Partition chromatography on paper of the material and of an authentic sample of aspartic acid, followed by development with ninhydrin solution, gave identical $R_F$ values for each sample.

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AMINO ACIDS DEPARTMENT,
THE BRITISH DRUG HOUSES LTD., LONDON, N.1. [*Received, May 30th*, 1952.]

# OBITUARY NOTICE.

## HENRY DRYSDALE DAKIN.

### 1880—1952.

H. D. DAKIN, an organic chemist who through his experimental and theoretical work contributed effectively to the establishment of biochemistry as an independent discipline, was born on March 12th, 1880, in London, whence his father, Thomas Burns Dakin, a merchant, soon moved to Leeds. Here he received all his early training; after attending Leeds Modern School, he worked for a brief period in the laboratory of Fairley, the City Analyst, and in 1898 he entered Yorkshire College, now the University of Leeds, to study chemistry under Julius B. Cohen. While still an undergraduate, he collaborated with that eminent chemist in an investigation of the halogenation of aromatic compounds under the influence of the aluminium–mercury couple. This led to systematic work on the constitution of the di-, tri-, and tetra-chlorotoluenes. In addition, Dakin's interest in analytical chemistry, no doubt stimulated by his experiences in the City laboratory, led him at the same time to study the use of phosphate in the determination of various metals and the applications of persulphate to the determination of nitrogen, manganese, and chromium.

In 1901 Dakin was granted the baccalaureate in Science of Victoria University, Manchester, of which Yorkshire College was then an affiliate. In the following year he was awarded an 1851 Exhibition scholarship which enabled him to work in other laboratories. The first of these was the Jenner Institute, in London, the title of which was soon changed to the Lister Institute of Preventive Medicine. Here he joined S. G. Hedin in a study of the proteolytic action of an enzyme present in kidney cells, and then began an independent investigation of the differential activity of lipase towards the respective stereoisomeric components of esters of racemic mandelic acid. In this work, which had an immediate effect on the theory of enzyme action, he showed (*J. Physiol.*, 1904, **30**, 253) that esters of the dextrorotatory acid are more readily hydrolysed by the enzyme than are those of the lævorotatory form. This led him to advance the view, now universally accepted, that the primary step in the process is the formation of a transitory compound of enzyme and substrate.

During 1904 Dakin worked in Heidelberg, where he collaborated with Albrecht Kossel in studies of protamines; these culminated in the discovery of arginase (*Z. physiol. Chem.*, 1904, **41**, 321). On his return to England he rejoined the staff of the Lister Institute, resuming his investigations of the action of lipase on mandelic esters and analogous compounds. In these (*J. Physiol.*, 1905, **32**, 199) he demonstrated the dependence of sensitivity to the enzyme on the configuration of the acyl group rather than on the direction of the optical rotation of the intact ester. He also observed selective enzymic hydrolysis of esters in which the potential asymmetry resided in the alkyl group, a confirmation of his previous conclusion that the enzyme combines with the intact esters before their hydrolytic breakdown. He also carried out a synthesis of epinephrine and allied bases, and related their physiological activity to chemical structure (*Proc. Roy. Soc.*, 1905, *B*, **76**, 491, 498).

In 1905 Dakin accepted an invitation from Christian A. Herter, a founder and benefactor of the *Journal of Biological Chemistry*, to work in a private laboratory which Herter had established in New York. In this environment Dakin was not only enabled but encouraged to devote his entire time to investigations of his own choice. His first publication after his arrival (*J. Biol. Chem.*, 1906, **1**, 171) concerned the oxidation of amino-acids in neutral solution by hydrogen peroxide in the presence of traces of ferrous salts, whereby deamination and decarboxylation occurred with formation of aldehydes (and acids) containing one carbon atom less. This reaction proved to be general, except in the case of glycine, which yielded notable proportions of glyoxylic acid. During the following years the action of this almost physiologically mild method of oxidation, which had previously been successfully employed by Fenton for the oxidation of sugars, was applied to a wide variety of organic substances of metabolic interest. Unlike glycine, sarcosine and glycollic acid were found to yield no glyoxylic acid; however, this was formed in traces from betaine and hippuric acid and readily from creatine and creatinine. Benzoates, studied in collaboration with Herter's daughter Mary, were shown (*ibid.*, 1907, **3**, 419) to become hydroxylated, primarily to salicylic and *p*-hydroxybenzoic acids, and secondarily to catecholcarboxylic acids.

These results stimulated studies of the oxidation of simple aliphatic compounds in the

animal body. Dakin showed that whereas the administration of acetate did not result in oxaluria, oxalic acid was excreted after the ingestion of glycollic acid or ethylene glycol. Glyoxylic acid, which also yields oxalic acid *in vivo*, was found not to be a precursor of allantoin.

His observation that acetone is an end-product of the oxidation of leucine by an excess of hydrogen peroxide led Dakin to his classical work (*ibid.*, 1903, **4**, 71, 91, 221, 227, 419; **5**, 173, 303) on the β-oxidation of fatty acids *in vitro* and *in vivo*. This elegant and exhaustive investigation supplied a satisfactory chemical explanation of the results secured four years earlier by Knoop in his study of the metabolic fate of terminally phenylated fatty acids. In the course of this work Dakin showed that the methyl ketones acetone and acetophenone, formed by decarboxylation of β-keto-acids, are degraded to acetic and benzoic acids in the living animal as well as by hydrogen peroxide *in vitro*. The question whether the primary products of the biochemical oxidation of fatty acids are β-hydroxy- or β-keto-acids was left open; Dakin showed, however, that when a β-hydroxy-acid is excreted it always appears as a lævorotatory form.

Incidentally to his experiments on the oxidation of organic compounds by hydrogen peroxide, Dakin introduced (*ibid.*, 1908, **4**, 235) the use of *p*-nitrophenylhydrazine for the characterization of aldehydes and ketones. He then discovered (*Proc.*, 1909, 194) a remarkable general reaction of aromatic aldehydes and ketones containing a phenolic hydroxyl group in the *ortho*- or *para*-position: for example, salicylaldehyde, when treated in faintly alkaline solution with hydrogen peroxide, breaks down into catechol and formate; *p*-hydroxyacetophenone similarly treated yields quinol and acetate.

A study of the metabolic fate of γ-phenylvaleric acid and its β-hydroxy-derivative, which gave rise to the excretion of hippuric acid, led Dakin to advance (*J. Biol. Chem.*, 1909, **6**, 221) the hypothesis that the catabolism of fatty acids involves the successive removal of two carbon groups at a time. The fact that benzoic and phenylacetic acids, the ultimate degradation products of ω-phenylated fatty acids containing respectively odd and even numbers of carbon atoms in the side chain, are excreted in the form of their glycine derivatives was correlated with the observation that conjugation with glycine diminishes the toxicity of phenylpropionic acid (*ibid.*, 1908—09, **5**, 173, 303; 1909, **6**, 203). Introduction of a hydroxyl group into the β-position of phenylpropionic acid had the same effect. Administration of β-hydroxy-β-phenylpropionic acid and of phenylglyceric acid led to the excretion of hippuric acid; on the other hand, racemic phenylalanine and phenyl-β-alanine were found to reappear in the urine in the form of their ureides without undergoing oxidative degradation (*ibid.*, 1909, **6**, 235).

In collaboration with A. J. Wakeman, Dakin demonstrated (*ibid.*, 1909, **6**, 373; 1910, **8**, 105) the presence in liver of enzymes which promote the reversible oxidation of β-hydroxybutyric acid to acetoacetic acid.

At about the same time, having shown (*Amer. Chem. J.*, 1910, **44**, 45) that optically active hydantoins (though not hydantoic acids) are rapidly racemised in dilute alkali, Dakin and Lafayette B. Mendel of Yale University demonstrated the optical inactivity of urinary allantoin, and suggested that this was due to enolisation (*J. Biol. Chem.*, 1910, **7**, 153).

He then threw some light on the chemical nature of alcaptonuria (*ibid.*, 1910, **8**, 11, 25; 1911, **9**, 151). Racemic tyrosine when administered to normal animals was found to be partly excreted in the form of its ureide and hydantoin, and its methyl ether yielded some *p*-methoxyphenylacetic acid. As tyrosine methyl ether and *p*-tolylpyruvic acid, neither of which is capable of forming a *p*-quinonoid derivative, were found to yield acetoacetic acid on perfusion through a surviving liver, Wakeman and Dakin (*ibid.*, 1911, **9**, 139) concluded that ring-opening is involved in the normal catabolism of tyrosine. An alcaptonuric subject was observed to be able to effect the complete oxidation of *p*-tolyl- and *p*-methoxyphenyl-alanine; the primary abnormality in alcaptonuria was therefore thought to consist in the formation of homogentisic acid rather than a failure to oxidise it.

With Wakeman, Dakin studied (*ibid.*, 1911, **9**, 327) the then obscure biochemical relation between urea and ammonia; as perfusion of urea through a surviving liver gave rise to no ammonia at the expense of urea, it was concluded that the postulated conversion *in vivo* of ammonium carbonate into urea was irreversible.

In a series of studies (*ibid.*, 1911, **9**, 139; 1912, **10**, 499; **13**, 513; 1913, **14**, 321) of the intermediary metabolism of amino-acids, Dakin, partly with Wakeman, extended Embden's observations according to which these compounds fell into two classes, those which are biochemically converted into glucose and acetoacetic acid respectively. It is noteworthy that he suggested the close metabolic relation, now firmly established through studies made with the aid of isotopes, of proline to ornithine and glutamic acid.

In 1913, Dakin was joined by the late Harold W. Dudley, also one of the impressive number

of brilliant students of J. B. Cohen who became leaders in the field of biochemistry. This collaboration, though it lasted only two years, was a singularly fruitful one. Their first joint publication (*ibid.*, 1913, **14**, 423) recorded the discovery of an enzyme, widely distributed among mammalian tissues, which converts methylglyoxal and phenylglyoxal into lævorotatory lactic and mandelic acid respectively. This enzyme, glyoxalase, stable to alkali but inhibited and inactivated by acid, was shown to be present also in fish, oysters, and toads, but not in green plants or (except for traces) in yeast. Red blood cells were found to be especially rich in the enzyme. On the other hand, pancreas not only yielded no glyoxalase but contained a specific, thermolabile antiglyoxalase; abdominal lymph glands contained neither the enzyme nor the inhibitor. The enzymic reaction appeared to be general for α-keto-aldehydes; for example, glyoxalase was found readily to convert glyoxal into glycollic acid. Glyoxalase was shown to differ from Neuberg's keto-aldehyde mutase, which had no effect on glyoxal, and from the aldehyde mutase of Parnas, which was not inhibited by pancreas extract. Incidentally, pancreatic antiglyoxalase was found to have no effect on the production of acetoacetate from butyric acid or tyrosine by perfusion through the liver (*ibid.*, 1913, **15**, 463; 1914, **16**, 505, 515; *Biochem. Z.*, 1914, **59**, 193).

In studies stimulated by the idea that α-keto-aldehydes may represent a cardinal phase in the metabolism of carbohydrates and amino-acids, Dakin and Dudley (*Proc.*, 1913, **156**, 192; *J. Biol. Chem.*, 1913, **14**, 555; **15**, 127) discovered that when lactic acid and alanine are kept in dilute solution at 37° in the presence of *p*-nitrophenylhydrazine, the *p*-nitrophenylosazone of methylglyoxal is deposited. Analogous reactions were observed with glycollic, glyceric, and mandelic acids; also with glycine, leucine, and other amino-acids. The chemical mechanism of this remarkable reaction (Dakin, *Biochem. J.*, 1916, **10**, 313) is still obscure, and its significance in intermediate metabolism has yet to be established. By elaborate and painstaking studies, Dakin and Dudley conclusively demonstrated the metabolic formation of optically active amino-acids from glyoxals.

An outcome of Dakin's earlier work on the racemisation of hydantoins had been a preliminary study (*J. Biol. Chem.*, 1912, **13**, 357) of the action of alkali on gelatin. His theoretical prediction that the non-terminal amino-acid groups in peptide chains would lose their rotatory power was confirmed; of the individual amino-acids isolated from a hydrolysate of alkali-treated gelatin, some were optically unchanged, some partly racemic, and some optically inactive. In a laborious investigation of " racemised " casein, Dakin and Dudley (*ibid.*, 1913, **15**, 263, 271) showed that of the eleven component amino-acids isolated one, namely proline, retained its full activity and must therefore have been present in only terminal positions in the protein molecule. Of the remainder, three (alanine, valine, and leucine) were partly racemic and must therefore have been represented among the terminal units; the others were completely inactive. " Racemised " gelatin, on the other hand, yielded fully active glutamic acid, lysine, and proline, partly racemic alanine, and inactive leucine; all other amino-acids isolated were completely racemic. Apart from its altered optical activity, the alkali-treated casein closely resembled native casein in physical properties; it was, on the other hand, entirely unaffected by pepsin, trypsin, and erepsin, and when fed to a dog was excreted unchanged in the fæces.

Other studies published jointly by Dakin and Dudley (*ibid.*, 1914, **17**, 29, 451, 275; *J.*, 1914, **105**, 2453) related to a method for the resolution of α-uramino-acids by means of their strychnine salts; a demonstration of the metabolic conversion of the " unnatural " variety of alanine into glucose; some limitations of the Kjeldahl method for the analysis of cyclic compounds containing nitrogen in the ring; and a general method for the synthesis of alkylglyoxals from γγ-diethoxyacetoacetic ester.

During 1914 Dakin published a report (*J. Biol. Chem.*, 1914, **18**, 91) on the formation of benzoylcarbinol and L-mandelic acid from phenylglyoxal through the agency of active fermenting yeast. Early in 1915 he recorded (*J.*, **107**, 434) an attempt to resolve 3-methylallantoin into optical isomers, the failure of which supported the view that enolisation, rather than the transitory formation of a bicyclic structure, is responsible for the apparent lack of asymmetry in allantoin.

When war broke out in 1914, Dakin at once returned to Britain, but finding in his own country no immediate outlet for his ambition to aid in the war effort, he joined a research group at a French military hospital at Compiégne, where a study of methods for the antiseptic treatment of wounds had been organized by the late Alexis Carrel of the Rockefeller Institute for Medical Research in New York. Here he developed the hypochlorite solution which bears his name. By the use of borate buffer, he succeeded in greatly reducing not only the instability but also the irritant action of commercial hypochlorites. His review articles on the use of anti-

septics in the treatment of infected wounds and on the history of hypochlorites (*Brit. Med. J.*, 1915, II, 318, 809) are minor classics. In a note communicated to the French Academy of Sciences in August, 1915, and soon thereafter published (*Compt. rend.*, 1915, **161**, 150), he made generally available the details of the preparation of Dakin's solution. In this note he also referred to the possibilities of sodium salts of aryl-*N*-chlorosulphonamides, which had been investigated in 1904 by his former professor, J. B. Cohen. In an extensive study of compounds of this class, the results of which were published early in 1916 by Dakin, Cohen, Daufresne, and Kenyon (*Proc. Roy. Soc.*, 1916, *B*, **89**, 232), sodio-*N*-chlorotoluene-*p*-sulphonamide (chloramine-T) was found to be the most effective member of a long series tested. The mode of action was investigated, with amino-acids as models, in the Department of Biochemistry and Pharmacology of the Medical Research Committee (*Biochem. J.*, 1916, **10**, 313, 319; 1917, **11**, 79). With one molecular equivalent of the chloro-amide the initial product proved to be a chloro-amino-acid, which subsequently broke down into an aldehyde, carbon dioxide, and ammonia; with two equivalents, a dichloro-amino-acid was first formed and this decomposed into a nitrile and carbon dioxide.

At the time of the attempted invasion of the Dardanelles, Dakin was invited by the military authorities to assist them in their attempt to combat the infections prevalent among the wounded who were being evacuated. Through his personal efforts an electrolytic unit for the preparation of ample quantities of hypochlorite solution from sea-water was installed in the *Aquitania*, which was being employed as a hospital ship. A great and immediate reduction in the incidence of infections on board resulted. When the ship resumed her normal passenger trade, this unit was retained as part of her regular equipment and remained in use throughout her years of service.

With Major E. K. Dunham of the U.S. Army Medical Service, Dakin also developed (*Brit. Med. J.*, 1917, I, 682) the use of chloramine-T for the disinfection of drinking water, and noted that this agent was more effective when the water was weakly acidified.

While in the laboratory of the Medical Research Committee, Dakin joined the late George Barger in a study of the metabolic fate of ingested glyoxalinealdehyde and in an attempt to synthesize urocanic acid (*Biochem. J.*, 1916, **10**, 376). His important paper " On Amino-Acids," was published in 1918 (*ibid.*, **12**, 290); as it was entitled " A Report to the Medical Research Committee " it seems that the experimental work therein described was, at least in part, carried out in the same laboratory. His discovery that the monoamino-monocarboxylic acids are selectively extracted from neutral aqueous solutions by wet butanol not only contributed a valuable technique in the analytical isolation of the component amino-acids of proteins but formed the basis of essential features of some of the modern chromatographic methods for their separation. This work led to the isolation of a new amino-acid, hydroxyglutamic acid, the relation of which to glutamic acid was established by reduction. However, the precise structure of this acid, the properties of which (*ibid.*, 1919, **13**, 398) did not conform entirely with those deducible from the characteristics of optically inactive β-hydroxyglutamic acid synthesized at the time by Dakin and nine years later by Harington and Randall, is still uncertain. Indeed, some—though not all—attempts in other laboratories to confirm its very existence were unsuccessful.

After returning to New York, Dakin collaborated, across the Atlantic, with H. H. (now Sir Henry) Dale in a comparative study (*Biochem. J.*, 1919, **13**, 248) of the chemical structure and antigenic specificity of the crystalline ovalbumins of the domestic hen and of the duck. Dale's experiments indicated that these two proteins, which were apparently identical in amino-acid composition, could be distinguished from each other by anaphylactic tests; Dakin's work, carried out with the aid of his new procedure on hydrolysates of the " alkali-racemised " albumins, showed definite differences in the optical activity of the leucine, histidine, and aspartic acid.

Another important application of the use of partially miscible solvents to the separation of amino-acids was the quantitative isolation of the constituent units of gelatin (*J. Biol. Chem.*, 1920, **44**, 499) and zein (*Z. physiol. Chem.*, 1923, **130**, 159). Dakin's figures for the amino-acid composition of these proteins were the first to give an approximately complete accounting. His initial failure to isolate valine from zein led to a repetition of the work with a hydrolysate prepared by T. B. Osborne, whose previous report of the presence of valine in that protein was thereby confirmed (*J. Biol. Chem.*, 1924, **61**, 137).

During the early twenties Dakin devoted much of his time to the synthesis, resolution, and biochemistry of amino-hydroxy-acids, the action of muscle tissue on fumaric, maleic, malic, and glutamic acids, and the catabolism of fatty acids. His experimental findings (*ibid.*, 1922, **52**, 183; 1923, **56**, 43; 1924, **61**, 139) undoubtedly contributed much to current views on the carbohydrate cycle and its relation to the metabolism of fats and proteins. In 1922 he was

joined for a brief period by C. R. (now Sir Charles) Harington, with whom he studied the anomalous behaviour of α-diketones in the Strecker reaction, which was found to induce scission of the linkage between the carbonyl groups (*ibid.*, 1923, **55**, 487).

In 1926 he subjected thyroxine to chemical study and soon clarified its constitution. He submitted a paper on his results to the *Journal of Biological Chemistry*, but on learning that Harington had independently reached essentially the same conclusions he withdrew his manuscript from publication. In the same year, in collaboration with Eleanor B. Newton and the late Stanley R. Benedict of Cornell University (*Science*, 1926, **64**, 602; *J. Biol. Chem.*, 1927, **72**, 367), he identified thiasine, a constituent of blood, with ergothioneine, isolated from ergot by Tanret in 1909, the constitution of which had been established in 1911 by Barger and Ewins.

Two years later he and Randolph West, a young clinician, recorded (*J. Biol. Chem.*, 1928, **78**, 91, 745, 757) a novel reaction of α-amino-acids consisting in the conversion of these, by the action of acetic anhydride in pyridine, into carbon dioxide and the corresponding α-acetamidoalkyl methyl ketones. He then took up a study of the condensation of aromatic aldehydes with amino-acids, acylamino-acids, peptides, and proteins (*ibid.*, 1929, **82**, 439; **84**, 675), but soon abandoned this line of work in favour of a laborious attempt to isolate the hæmopoietic factor present in liver. The pursuit of this compound, which has only recently been isolated (vitamin $B_{12}$) and has not yet been fully characterized, was beset with insurmountable difficulties. At that time the only method for assaying the potency of preparations consisted in clinical tests on human subjects suffering from pernicious anæmia. These tests were tirelessly performed by Randolph West; however, the general application of liver therapy made it increasingly difficult to find suitable untreated patients. Secondly, the unpredecentedly high potency of the vitamin, then unrecognized, constituted a major pitfall in that any fraction which contained it as a difficultly separable contaminant had to be regarded as a concentrate. Thirdly, the precision of the clinical assay was low. Among the false leads pursued by Dakin and West were an acidic peptide which yielded hydroxyglutamic acid and hydroxyproline on hydrolysis; a tribasic acid containing the pyrrolidone ring; and a polypeptide containing a hexosamine in combination (*ibid.*, 1931, **92**, 117; 1935, **109**, 489; 1936, **115**, 771; *Proc. Soc. Exp. Biol. Med.*, 1939, **40**, 124).

During his last ten years, Dakin undertook only minor problems which had presented themselves in the course of his earlier studies; the synthesis of β-aminovaleric and β-methylaspartic acids; the production of betaines from amino-hydroxy-acids (in the course of which he recorded his doubt that the hydroxyglutamic acid which he had isolated from protein hydrolysates was a β-hydroxy-derivative); the co-precipitation of lysine and ornithine with silver hydroxide; the synthesis of γ-hydroxyleucine; the formation of γ-methylproline in an attempted synthesis of δ-hydroxyleucine. There is little doubt that the exacting and discouraging search for the hæmopoietic factor had taxed his health, never very strong, more than he was willing to admit.

Dakin loved to work with his own hands and avoided opportunities to direct the activities of research groups. The younger biochemists who had the privilege of working under him, and of thereby assimilating his scientific ideas and learning his experimental methods, were therefore few, but included at least two who subsequently became leaders. It is characteristic of him that he personally carried out all his analytical work, including combustions. He was probably the only organic chemist who consistently employed Barger's method for the determination of molecular weight. Incidentally, his knowledge of biochemical techniques was most successfully applied, as a result of Herbert Hoover's " noble experiment," to the annual preparation of wines of admirable quality.

Dakin wrote with ease and elegance. He was the author of a Monograph on Biochemistry entitled " Oxidations and Reductions in the Animal Body " which appeared in 1912 and again in 1922, and of Physiological Oxidations (*Physiol. Reviews*, 1921, **1**, 394). He was co-author, with his kinsman E. K. Dunham, of a small " Handbook of Chemical Antiseptics " (1917).

On the other hand, his horror of public speaking kept him from scientific conventions and other occasions on which he might have become more widely known to his scientific colleagues in person. His circle of friends, all of whom were devoted to him, was not large but included men of a wide variety of professions. To them all, he was known as " Zyme."

Many honours came his way. His own University of Leeds, which had granted him the Ph.D. in 1907, conferred on him the honorary Doctorate of Laws in 1936; Yale University gave him the honorary Doctorate of Science in 1918; Heidelberg University awarded him the Doctorate in Philosophy, *honoris causa*, about 1938. At the close of the first World War, the French Government made him a Chevalier of the Legion d'Honneur. In recognition of his chemical contributions to medicine, he was awarded the Conné Medal of the New York Chemists' Club about 1933; in 1942 he received the Davy Medal of the Royal Society.

In 1901 he was admitted to the Chemical Society and, as an Associate, to the Institute of Chemistry, of which he became a Fellow in 1904. He was elected to the American Society of Biological Chemists soon after its inception, and to Fellowship in the Royal Society in 1917.

Throughout the period 1909 to 1930 he was a member of the Editorial Board of the *Journal of Biological Chemistry*, which had been generously endowed by Christian A. Herter, and for many years he managed the finances of that *Journal* with a degree of efficiency rarely found among scientists. In his later years he served on the Board of Scientific Advisers of the Merck Institute of Therapeutic Research and ultimately became a Director of Merck and Co.

After the death of Dr. Herter in 1910, Dakin had, at the desire of Mrs. Herter, continued to work in the laboratory in New York City until his temporary return to Europe to serve in war work. In 1916, he and Mrs. Herter married. Two years later, after the Armistice, they moved to an estate in rural Scarborough-on-Hudson, N.Y., where he converted an adjacent building into a commodious laboratory and scientific library. Here, with the assistance of a family retainer who served as his laboratory " Diener," he carried on his researches until the end. His marriage was an ideally happy one. His wife's death in 1951, after a long illness during which he tended her with the utmost devotion, seemed to leave him with little desire to continue alone. His own death came peacefully, in his sleep, on February 10, 1952. He had no children.

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